The
AMERICAN JOURNAL
of
MEDICAL TECHNOLOGY

VOLUME 23 JULY-AUGUST, 1957 NUMBER 4

METHOD OF COUNTING THROMBOCYTES*

SISTER M. DANILE KEILY, B.S., M.T. (ASCP)
Garrison Memorial Hospital, Garrison, North Dakota

Apparatus

1. *Paraffin Cup*—Make a small paper box about ¾ inch square. Melt paraffin, pour into paper box, allow to harden. When the paraffin has hardened, remove the paper from the cube. Warm the end of a pyrex 13 x 100 mm. test tube over a bunsen burner and melt the center of the paraffin cube so that there is a depth of about ⅛ inch.
2. *Waxed Tooth Picks*—Dip tooth picks into warm paraffin and allow the paraffin to harden on them.
3. *Waxed Capillary Tubes*—Draw hot paraffin into 0.4 mm. or 0.8 mm. capillary tubes. Blow out immediately leaving a thin coating of paraffin in the tube.
4. *New and scrupulously Clean 3 x 1 inch slides.*

Reagents

1. *3.5% Sodium Citrate*—Dissolve 3.5 grams of C.P. Sodium Citrate in 100 ml. of Baxter's Distilled Water. Small amounts of this may be kept in a dropper bottle on the blood collecting tray ready for use.
2. *Hayem's or Gower's Solution* for the Erythrocyte Count.

Test Procedure

1. Place about 4 drops of 3.5% Sodium Citrate into the paraffin cup. The number of drops will depend upon the size of the dropper but should be a sufficient number to give a dilution of 4 parts of citrate to 1 part of blood.
2. Draw venous blood from the patient (release the tourniquet when the vein is entered). Collect about 2 cc. of blood or more if other tests are ordered.
3. Allow a few drops of blood to drip from the tip of the needle onto a piece of gauze or cotton, then insert the needle into the sodium citrate in the paraffin cup. Dispel about 1 drop of blood into the sodium citrate.
4. Mix the blood and sodium citrate by stirring gently with a waxed tooth pick.
5. Take an erythrocyte count on the blood.
6. Draw a small amount of the blood and citrate mixture into a waxed

* Read by title—Annual Convention, Chicago, Illinois, June 1957

capillary tube and place a very small drop of the mixture onto a new and clean slide. Spread in a thin film on the slide using another new, clean slide. We make the slide thin enough so that about 30 to 50 erythrocytes are seen per field under oil immersion. Make at least 2 smears in this manner.

7. Stain the smears with Wright's stain as for a differential.

8. Count the number of erythrocytes and thrombocytes per oil immersion field. We keep count of the erythrocytes with a hand tally counter and mark the number of platelets seen per field on a piece of paper. Continue to count fields over various parts of the slide until 1000 erythrocytes have been counted. 500 erythrocytes may be counted on each slide and the number of thrombocytes seen recorded.

9. Do the erythrocyte count to determine the number of erythrocytes per cu. mm.

10. Add the total number of platelets (thrombocytes) counted per 1000 erythrocytes and multiply by the number of thousands of erythrocytes per cu. mm. (first 4 numbers of the erythrocyte count).

Example: 70 thrombocytes counted per 1000 erythrocytes.
4,500,000 erythrocytes per cu. mm.
$70 \times 4{,}500 = 315{,}000$
Thrombocyte Count = 315,000 per cu. mm.
Normal Values—250,000 to 350,000 per cu. mm.

NOTE: Capillary blood may also be used. When capillary blood is used, cleanse the finger with surgical soap solution. Remove the soap with 10% acetone in 70% alcohol. Puncture the skin with a hemolet deep enough so the blood will flow freely without squeezing. Turn the finger so it is setting over the paraffin cup and let a drop of blood fall into the sodium citrate solution. Continue as in Step #4 above.

Comment

Thrombocytes are difficult to count. They are difficult to recognize in methods employing the hemocytometer. They are easily recognized but difficult to count in the direct smear methods. A method has been devised whereby the blood is diluted prior to making the smear. The thrombocytes appear as on a direct smear so are easily recognized. The thrombocytes are easily counted due to the dilution of the erythrocytes.

MICROHEMATOCRIT VERSUS ERYTHROCYTE COUNT*
I. CORRELATION OF MICROHEMATOCRIT, ERYTHROCYTE COUNT AND HEMOGLOBIN
II. SUGGESTED NORMAL RANGE FOR ERYTHROCYTES, HEMOGLOBIN AND PACKED CELL VOLUME FOR SOUTHERN OHIO

JOHN JOHNSON, M.T. (ASCP)
Supervisor, Diagnostic Laboratories, Miami Valley Hospital, Dayton, Ohio

The standard method for determining the state of the erythrocyte concentration in the blood is the erythrocyte count. This procedure requires the use of high dilutions of very small samples of blood taken into special capillary pipettes. The blood sample is well mixed with diluting fluid and the mixture placed in a counting chamber. The number of cells present in the dilution is ascertained, and by the use of a factor, the number of erythrocytes per cu. mm. of whole blood is estimated. Because of the large coefficient of variation found in this test under routine conditions, the value of a single erythrocyte count is very limited as an indication of a rise or fall in the erythrocyte content of the blood.

Various methods using the principles of opacity,[25,26] defraction,[6,21] photoelectric counting, and change in electric current[5] have been proposed to remove some of the inaccuracies, fatigue and time-consuming properties of the erythrocyte count. Unfortunately, these other methods still present difficulties which include eye counts for the calibration of photometers, the expense of complex electronic equipment, and the continued use of high dilutions of very small samples.

The hematocrit, on the other hand, has been shown to be highly reproducible;[4,19] it requires no dilutions and takes little time to prepare. It has also been shown to have an excellent linear correlation to the number of erythrocytes present in a normal sample of blood.[11]

The object of this paper is to show that a linear correlation exists between the packed cell volume obtained by a simple microhematocrit method, the erythrocyte count, and the hemoglobin content of the blood. From this, a plan for the substitution of the microhematocrit in place of the erythrocyte count in routine complete blood counts will be outlined. A normal range for the erythrocyte count, microhematocrit and hemoglobin for this section of Ohio, along with the erythrocyte constants and indices, will also be given.

Methods

The Blood Sample. Venous blood was used routinely in this investigation since McGovern et al.[19] have shown that the difference between the packed cell volume of capillary blood and that of venous blood determined by the microhematocrit are identical for all practical purposes. All blood samples were taken into "Vacutainers"† measuring 4" x ½" and containing 6 mg. ammonium oxalate and 4 mg. potassium oxalate as a dry powder. Five ml. of blood were taken from the arm with a

* Hillkowitz Award. Read before the Annual Convention, Chicago, Illinois, June, 1957.
†Manufactured by Becton, Dickinson and Company, Rutherford, New Jersey.

minimum of stasis. The blood was gently mixed with the anticoagulant by turning the tube end over end about twelve times. All samples of blood used in this study were checked for red cell morphology by examining a smear stained by Wright's method. Only those bloods having normal morphology were used. Before removing samples for testing, each tube was well mixed by turning gently end over end at least thirty times to insure a homogeneous suspension of the cells. Further mixing by inverting the tube two or three times between the removal of each sample, maintained a homogeneous suspension.

The Erythrocyte Count. Standard Thoma pipettes, taken from stock were used to dilute the blood for counting. Using Gower's[14] diluting fluid, a dilution of 1 in 201 was made. The erythrocytes were counted in 80 small squares on a Haden-Hauser hemocytometer with the improved Newbauer ruling, using a National Bureau of Standards certified cover slip. The number of erythrocytes per cu. mm. of whole blood was calculated by multiplying the number of erythrocytes counted by 10,000. Only one count from each pipette was done for the evaluation since this is the routine practice in this laboratory. The adoption or rejection of a count was determined by the criteria suggested by Berkson et al.[3]

The Hemoglobin Estimation. The cyanmethemoglobin method of Drabkin[9,10] was adapted to the Coleman Junior Spectrophotometer, model #6A‡ set at a wave length of 540 mu. The cyanmethemoglobin reagent was used as the reference solution. The calibration curve was prepared, using three dilutions of a cyanmethemoglobin standard supplied by the College of American Pathologists.

Matched Coleman grade B cuvettes, measuring 105 mm. in length by 199 mm. in diameter, were used.‡

The blood was taken up in stock 20 cu. mm. Sahli pipettes, the outside of the pipette wiped free of blood and then placed into 10 cc. of reagent in a test tube giving a dilution of 1 to 500. The blood in the pipette was washed out by raising and lowering the reagent in the pipette about five times. The tube was stoppered, the contents well mixed, and then poured into the cuvette. The transmittance was noted and grams hemoglobin per 100 ml. of blood was read from a calibration chart.

The Microhematocrit. The centrifuge used in this study was an International Microhematocrit, model #MH,* capable of a speed of 11,000 r.p.m., giving a relative centrifugal force (R.C.F.)[15] at the middle of the filled capillary tube of about 9,000 × G at full speed.

Commercially obtained capillary tubes of soft glass, containing no anticoagulant, 75 mm. in length and 1.3 to 1.5 mm. O.D.† were used to contain the well mixed blood sample. These tubes were filled to about 2 cm. from the top by capillarity, as recommended by Guest et al.,[12] the outside then wiped clean of blood and the opposite end closed to form a flat base by rotating slowly while heating in the periphery of a Blue Bunsen Burner flame. The filled tubes were centrifuged for four minutes. The capillary tubes were removed from the head immediately after

* Manufactured by International Equipment Company, Boston, Massachusetts.
† Obtained from American Hospital Supply Corporation, Scientific Products Division, Evanston, Illinois.
‡ Manufactured by Coleman Instruments, Inc., Maywood, Illinois.

centrifuging and placed upright in a rack. The packed cell volume was read directly on an International Spiral Microhematocrit Reader* according to the method of Jones.[16]

The Evaluation of the Erythrocyte Count, Microhematocrit and Hemoglobin Content

Several samples of venous blood of varying packed cell volume were taken and duplicate erythrocyte counts, microhematocrits and hemoglobin estimations were prepared on each sample by several technologists and processed as explained in the procedures above. The results were tabulated and the standard deviation, 95 percentile deviation, deviation at normal range and deviation at anemic range were calculated. These results are shown in Table 1.

TABLE 1

Comparison of the Standard Deviations of the Erythrocyte Count, Hemoglobin and Packed Cell Volumes

TEST	Number of Observations	Standard Deviation	95 Percentile Deviation	Deviation at Normal Range	Deviation at Anemic Range
Erythrocyte Count	203	0.248	±0.49 million per cu. mm.	±11.0%	±16.0%
Hemoglobin Content	180	0.221	±0.44 grams per 100 ml.	± 3.0%	± 4.0%
P.C.V. of 5 cm. Blood Column	78	1.52	± 3.0 units	± 7.0%	±10.0%
P.C.V. of 2 cm. Blood Column	133	0.51	± 0.98 units	± 2.0%	± 3.0%

As will be noted from this table, the deviation of the erythrocyte count and hemoglobin estimation are in close agreement with the generally accepted standards for clinical laboratories.[4, 5, 18] The microhematocrit, on the other hand, produced a deviation far greater than was desirable and much greater than that noted in the literature.[12, 19]

The variation in the microhematocrit result may be due to one or more of the following:

1. Unevenness of the bore of the capillary tube.
2. Unreliable reading methods.
3. Insufficient centrifuging time.
4. Loss of carbon dioxide, evaporation of fluid from the blood while standing or centrifuging, or both.
5. Changes in cell volume caused by the anticoagulant.

Experiments were arranged to test these points and endeavor to find the cause of the large variation found in the microhematocrit results and to reduce it if possible.

The Length of the Blood Column. If there is an unevenness of bore in the capillary tubes, then the longer the column of blood, the more the uneveness will be reflected in the variation of the packed cell volume. In order to investigate the effect of shortening the blood column, three sets of twelve capillary tubes were filled from a Vacutainer containing a well

* Manufactured by International Equipment Company, Boston, Massachusetts.

mixed sample of normal blood. The first set had a blood column 5 cm. in length, the second set 3 cm. in length, and the third set 2 cm. in length. All tubes were sealed and centrifuged for four minutes. The tubes were filled and read by the same technologist, thus keeping any personal error constant. The results were tabulated and the standard deviation and 95 percentile deviation were calculated. The results are shown in Table 2. It will be noted that the 95 percentile deviation had been reduced four times by reducing the height of the blood column, giving an indication that uneveness of bore was at least partially responsible for the large variation in results previously obtained.

TABLE 2

The Effect of the Length of the Blood Column on Microhematocrit Variance

Length of Blood Column	Number of Observations	Average P.C.V. Per cent	Variance	95 Percentile Deviation
5 cm.	12	36.90	3.4159	±4.13
4 cm.	12	36.05	2.0155	±3.17
2 cm.	12	35.16	0.2391	±1.09

To Find the Variance Due to Reading. By having four technologists independently read the three sets of tubes from the last experiment, the variance due to reading was established. The reading variance was found to be constant in all three sets of microhematocrits and amounted to 0.0987, giving a 95 percentile deviation of ± 0.5 units. This amounts to half of the total variance found for the 2 cm. column of blood.

A statistical test (F test)[13] applied to the variances for all three sets of data showed that they came from the same distribution and could, therefore, be pooled to obtain a better estimate of the variance due to reading error.[13] This proved to be 0.078, the standard deviation was 0.279 and the 95 percentile deviation was plus or minus 0.55 hematocrit degrees.

Another interesting fact derived from these experiments was the apparent decrease in packed cell volume in spite of the fact that all three sets of capillary tubes were filled from the same sample of blood, centrifuged for the same length of time and at the same constant speed.

The shortened cell column causes the effective radius of the centrifuge (measured from the center of the head to the middle of the blood column) to be increased from 6.5 to 8.5 cm., thus the average R.C.F. exerted on the 2 cm. blood column is raised from 9,000 × G to 11,000 × G at 11,000 r.p.m. The higher R.C.F. causes a reduction in the amount of plasma trapped between the cells reducing the packed cell volume in accordance with the experiments of Armstrong et al.[1]

The Centrifugation Time. In order to check the time required to achieve a constant packing and to determine if incomplete packing could account for some of the wide variation, two samples of blood, one in the high packed cell volume range and one in the low range, were used.

Twenty capillary tubes containing a 2 cm. column of blood were prepared from each of the two samples. After sealing in the flame, four tubes from each set were centrifuged for one, two, three, four and five minutes. The machine was timed from the moment the power was

switched on to the instant the power was switched off. The time required for the machine to stop after the power was turned off amounted to one minute and thirty-six seconds in each case.

Figure 1 illustrates the curve formed when the average of the packed cell volumes of each group of four tubes was plotted against the time in minutes. It will be noted that the low range sample packed to constant volume in two minutes. Three minutes, timed with the automatic timer attached to the centrifuge, was sufficient to provide constant packing for samples of high packed cell volume. Therefore, four minutes of centrifugation time provides an ample margin of safety for the constant packing of erythrocytes in blood samples over the normal range.

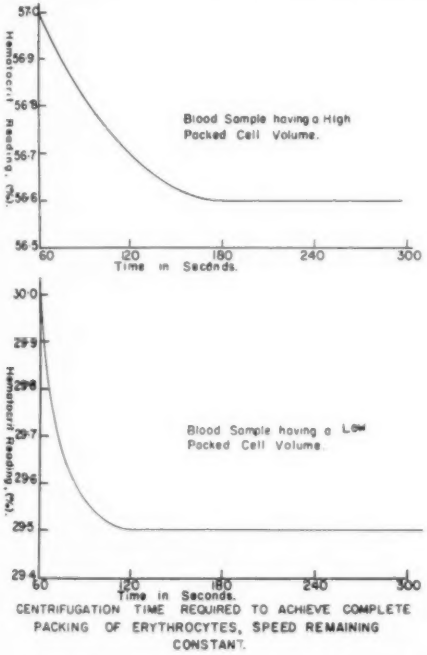


Figure 1
Microhematocrit v. Erythrocyte Count.

The Effect of Evaporation of Water and Loss of Carbon Dioxide. According to Smirk,[27] the loss of carbon dioxide and water from the microhematocrit causes considerable variation in the packed cell volume. He recommended that the column of blood be protected from evaporation and loss of carbon dioxide by a layer of mineral oil.

In order to determine whether part of the large variance in our microhematocrit results could be due to these factors, two freshly drawn, well mixed samples of blood were used. Microhematocrits were prepared immediately. In one set of twelve the column of blood was left in contact with the air at the top of the tube; in the second set of twelve the blood was allowed to run down into the tube for a distance of 1 to 2 cm.; and

in the last set a small drop of mineral oil was added. The distal end of the tubes was sealed in the flame.

Two tubes from each set were centrifuged immediately at 11,000 r.p.m. for three minutes, two others from each set were centrifuged at fifteen minute intervals over a one hour period, the last two tubes from each set were centrifuged after ninety minutes. All tubes remained at room temperature while waiting to be processed. The values of each pair were averaged and the averages recorded.

The tubes with blood left in intimate contact with the air had dried across the top and become useless in fifteen minutes. No significant difference was found between the averages of the other two sets even after standing for ninety minutes. The maximum difference in average microhematocrit readings, between the set having oil added and the set with the blood surface 1 to 2 cm. below the opening of the tube, was only 0.3 hematocrit units.

Effect of Anticoagulant and Time on the Packed Cell Volume. Two samples of normal blood were drawn into Vacutainers containing the double oxalate anticoagulant, mixed well and quadruple microhematocrits prepared at once from each tube. The stoppers were immediately replaced in the Vacutainer to avoid any possible evaporation of water over the long period of standing at room temperature. The microhematocrits were sealed and centrifuged for three minutes, readings of the packed cell volume were taken and the averages recorded. The procedure was repeated every hour for a period of four hours, on the same blood samples.

The maximum difference found was only 0.3 hematocrit units, and it was, therefore, concluded that the anticoagulant causes no significant changes in cell size over a four hour period and that the double oxalate would be an ideal anticoagulant for this study.

From these experiments a modified procedure for the estimation of packed cell volumes by microhematocrit was prepared. The use of a 2 cm. column of blood was instituted while the time of centrifugation remained at four minutes. The 2 cm. column of blood was allowed to run into the tubes for a distance of between 1 and 2 cm., the distal end sealed at once and the sample centrifuged. The microhematocrits were removed from the centrifuge head as soon as possible and placed upright in a rack until read.

Estimation of Variance of New Hematocrit Procedure. The experiment to show the variance of the microhematocrit was repeated on 133 samples using the new procedure. The results are shown in Table 1.

The new procedure significantly reduced the variation and made the microhematocrit the least variable of the three tests.

The Construction of the Regression Lines for the Microhematocrit Reading Versus the Erythrocyte Count and Hemoglobin Estimation.

Blood was drawn, as stated in the section on Methods, from healthy adult male blood donor registrants, 19 to 60 years of age. Stained smears of each blood sample were scanned and those showing any erythrocyte abnormalities were not included in this study. One hundred and fifty samples of blood having erythrocytes of normal size, shape and hemoglobin content were collected.

Blood samples were drawn from the same age group of healthy female employees of the hospital and screened as before. Eighty samples showing normal erythrocyte morphology were used in the study.

In spite of the fact that no conclusive evidence has been found in the literature to show that a significant change in packed cell volume can be traced to normal menstrual bleeding,[7, 28, 34] all samples were taken two weeks after the menstrual period to avoid, as far as possible, any physiologic changes in the packed cell volume of the samples from the female population.

Both male and female blood samples were drawn in the morning just before lunch and after normal activity. For the purpose of calculating the regression lines both male and female results were pooled to give a total of 230 specimens. It was felt that this course was justified since there does not appear to be any significant difference in size or shape of the erythrocytes of the two sexes.

In order to keep the personal error constant, one technologist did all the experimental work in this study.

Since the standard deviation of the microhematocrit was found to be the smallest, it was chosen to be the independent variable (x axis) in the construction of the regression line, the erythrocyte count and hemoglobin results were chosen as the dependent variables (y axis).

Duplicate erythrocyte counts, microhematocrits and hemoglobin estimations were set up on each blood sample. The results were averaged and the averages of all three tests were recorded. A scatter diagram prepared from these results indicated a possible linear relationship between the microhematocrit and erythrocyte count and the microhematocrit and the hemoglobin estimation. Calculation of the correlation coefficient for both pairs of tests yielded a result greater than 0.9 indicating that the relationships were suitable for linear regression analysis.

TABLE 3

Summary of Results for Constructing Regression Lines and Calculating Confidence Limits for 230 Normal Adults

P.C.V. % (x axis)	Number of Samples	ERYTHROCYTE COUNT (y axis)		HEMOGLOBIN CONTENT (y axis)	
		Variance (S^2)	Average in millions/cu. mm.	Variance (S^2)	Average in Grams/100 ml.
37	5	0.0162	3.87	0.2831	11.85
38	6	0.0857	3.88	0.0800	12.40
39	13	0.0272	3.92	0.0975	12.70
40	15	0.0571	4.02	0.3439	12.74
41	12	0.1131	4.29	0.2073	13.30
42	21	0.0795	4.29	0.1900	13.60
43	20	0.0509	4.41	0.3412	13.83
44	27	0.0617	4.58	0.1131	14.60
45	27	0.0572	4.61	0.1717	14.56
46	22	0.0699	4.75	0.2977	14.95
47	17	0.0774	4.70	0.1469	15.40
48	20	0.0589	4.95	0.2831	15.71
49	10	0.1399	5.13	0.1422	15.94
50	11	0.0550	5.17	0.4690	16.03

Table 3 shows the variance and average erythrocyte count and hemoglobin content found for each microhematocrit value. Table 4 shows the parameters used to fix the slope of the regression line and the confidence

TABLE 4
Parameters Used for the Regression Lines and the Confidence Limit Calculation (13)

Microhematocrit (x) versus	a	b	Sa^2	Sb^2	Mean PCV% $(\bar{x})$
Erythrocyte Count (y) . . .	4.53	0.1	2.6×10^{-4}	2.5×10^{-5}	44.1
Hemoglobin Estimation (y)	14.31	0.32	9.0×10^{-4}	2.5×10^{-5}	44.1

Regression line formula is $y = a + b(x_i - \bar{x})$

Confidence limit formula is $Sy^2 = Sa^2 + Sb^2 (x_i - \bar{x})^2$

limits. All calculations were those given by Hald.[13] The packed cell volume units when plotted against the calculated erythrocyte and hemoglobin values on coordinate graph paper produced a regression line which indicated the form of the relationship between microhematocrit and erythrocyte count, and microhematocrit and hemoglobin content. Ninety-five per cent confidence limits were calculated for the two regression lines, the completed graphs are shown in Figures 2 and 3.

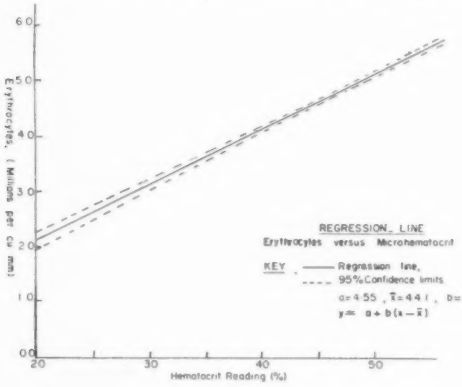


Figure 2—Microhematocrit v. Erythrocyte Count.

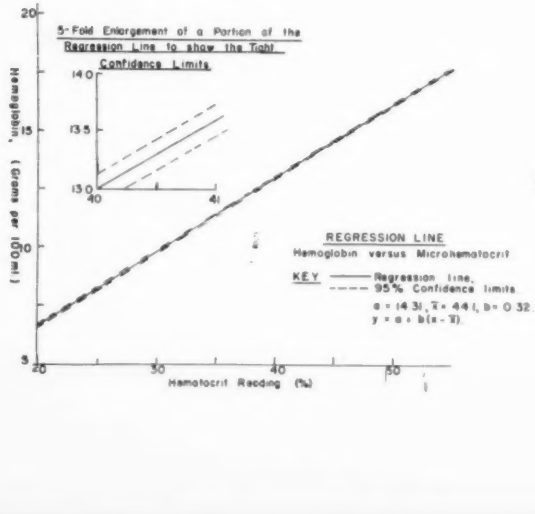


Figure 3—Microhematocrit v. Erythrocyte Count.

The Calculation of Normal Values

The average microhematocrit, erythrocyte count and hemoglobin concentration was calculated for the male and female population samples. The standard deviation was calculated and the normal deviations found by multiplying the standard deviation by two to give the approximate 95 per cent limits. The blood indices and constants were calculated according to the methods given by Wintrobe.[36] The values found are given in Table 5.

TABLE 5
Normal Values Estimated From 150 Males and 80 Females Aged 19-60 Years

	Erythrocyte Count Millions per cu. mm.	Hemoglobin Content Gm. 100 ml.	Micro P.C.V. %	M.C.V. cu. microns	M.C.H. Micro-micro-grams	M.C.H.C. cl. %	Hemoglobin Coefficient Gm. 100 ml.	C.I.	V.I.	S.I.
Male	4.70 ± 0.7	15.0 ± 2.0	46 ± 5	98 ± 10	32 ± 4	32 ± 3	15.6	1.0	1.0	1.0
Female	4.20 ± 0.6	13.0 ± 2.0	41 ± 5	98 ± 10	31 ± 4	32 ± 3	15.5	1.0	1.0	1.0

In the past it has been the practice to estimate adult normal values on a group of persons in the age group of 20 to 30. It was felt by the author that this may be misleading since the normal population contains a very large number of people whose age is above this value and yet are perfectly healthy and normal. Hence, the age range chosen for this study was from 19 to 60. The only condition required for a blood sample to be used was that the erythrocytes possessed normal morphology. This approach was justified when upon calculating normals for age group 19 to 30 and for age group 31 to 60 for both sexes, no significant difference was found between their means and variances.

Discussion

Over the past fifteen years there has been a tremendous increase in the volume of work performed by clinical laboratories. One of the most frequently requested procedures is the erythrocyte count which has changed little in technic since the time of its inception. The routine method is inaccurate, time-consuming and fatiguing to the technologist. To obtain an error of less than 8 per cent, specially calibrated expensive equipment is required, multiple counts must be made and the results averaged.[18]

The normal error to be expected from a single count made by a competent technologist has been shown by various investigators to vary from about 10 per cent to as high as plus or minus 16 per cent.[3, 4, 5, 18] This means that an erythrocyte count of five million per cu. mm. must show a variation greater than 1.6 million before the change can be considered significant. Our own expected standard deviation in the normal range of erythrocyte counts under routine conditions has proved to be on the order of 0.248 and this indicates that a five million erythrocyte count must increase or decrease more than 0.98 million erythrocytes before the change can be considered significant. The application of this standard deviation to counts in the anemic range increases the 95 percentile deviation so that a proportionately larger variation in the erythrocyte count is required before the change can be considered significant.

On the other hand, the macrohematocrit has been shown to be reliable

and if centrifuged at a sufficiently high speed has a very small coefficient of variation, the expected error being as small as three per cent of the packed cell volume. However, the method has two important disadvantages, namely, the macrohematocrit requires a comparatively large quantity of blood, and a long period of centrifugation time to pack the cells to constant volume.

The development of the microhematocrit, requiring only very small quantities of blood, began in 1909 when Bönniger[7] used a U-shaped capillary tube to estimate the packed cell volume of capillary blood. Acceptance of the microhematocrit for routine use was slow because of the long periods of time required to pack cells to a constant volume in centrifuges having comparatively low maximum speeds. The experimenters also found difficulty in making adequate seals for the capillary tubes. Small losses of fluid and cells around the beeswax plug or rubber sealer caused the methods to lack reproducibility.[24]

In 1931 Beams and Weed[2] used an ultra micro air turbine centrifuge capable of high speeds and obtained constant packing of suspended particles in a sealed capillary tube at a relative centrifugal force of one million × G in a matter of seconds. In 1943 this apparatus was adapted to the estimation of packed cell volume by Parpart and Ballentine.[20] Excellent reproducibility was obtained, less than two minutes was required for complete packing. Guest et al. in 1934[12] used a special micro head on an International Electric Centrifuge, and using a speed of 14,000 r.p.m. achieved complete packing of erythrocytes in five minutes. Strumia et al. in 1950[29] also adapted Beam's air turbine to the estimation of packed cell volume using the method of Parpart and Ballentine. Excellent reproducibility was obtained, the variation was found to be plus or minus 0.5 per cent. In 1954 Strumia[30] repeated his experiments using a specially designed ultra micro centrifuge capable of speeds up to 20,000 r.p.m. and having an R.C.F. of about 16,000 × G. These later experiments showed a much reduced variation of only plus or minus 0.21 hematocrit units. Complete packing was obtained in only two minutes.

Using an International Micro Centrifuge developed from that originally used by Guest and capable of a constant speed of 11,000 r.p.m., having an effective radius of about 8.5 cm. with a 2 cm. blood column giving an R.C.F. of approximately 11,000 × G, we achieved a constant packed cell volume in three minutes having a variation of only plus or minus 0.98 of a hematocrit unit.

The microhematocrit is, therefore, a rapid and highly reproducible method of estimating the number of erythrocytes of normal size and shape present in a given sample of blood. The reproducibility obtained is many times greater than that of the single erythrocyte count, making slight changes in the erythrocyte content of the blood easily detectable.

The microhematocrit finds its greatest use as a screening test in routine complete blood counts. Since a stained smear is always part of this procedure, the morphology of the erythrocytes is easily checked. The presence of anisocytosis, and/or poikilocytosis in erythrocyte morphology calls for an erythrocyte count; normal size and shape would indicate that a microhematocrit may be sufficient.

The comparatively small experimental error encountered in the hemo-

globin estimation coupled with that of the microhematocrit and the close linear correlation found between these two tests make it possible to use these two results to arrive at an estimated erythrocyte count when the size and shape of the erythrocytes are normal.

By using the ratio, mean erythrocyte count over mean packed cell volume, one hematocrit unit is equivalent to 102,000 erythrocytes per cu. mm. Thus a microhematocrit of 45 can be read as equivalent to an erythrocyte count of 4.59 million per cu. mm. Since the variation expected in the microhematocrit is plus or minus one unit, this count will be substantially correct within the limits of plus or minus 102,000 cells. This is a result well within total error of the erythrocyte count even when multiple counts are done and the results averaged.

Using the mean erythrocyte count and hemoglobin value from the regression line calculations as 100 per cent, and modifying the formula for color index, the following formulae were derived to fix limits to the hemoglobin values permissible for a given microhematocrit reading. The limits are based on the normal color index range of 0.9 to 1.1.

1. For the lower limit having a color index of 0.9 the formula is
$$y = 2.84\ x$$
2. For the upper limit having a color index of 1.1 the formula is
$$y = 3.47\ x$$

where x is the estimated erythrocyte count in millions per cu. mm. found by multiplying the microhematocrit reading by 0.102, and y is the grams of hemoglobin per 100 ml. of blood.

Table 6 shows the hemoglobin values calculated for this laboratory.

TABLE 6

Maximum and Minimum Hemoglobin Values for Given Microhematocrit Values From 35% to 54% to Give Color Indices Within the Range 0.9 to 1.1

P.C.V. %	Calculated Erythrocyte Count, Millions/cu. mm.	Minimum Hemoglobin Value, Grams/100 ml. C.I. = 0.9	Maximum Hemoglobin Value, Grams/100 ml. C.I. = 1.1
35	3.57	10.1	12.4
36	3.67	10.4	12.7
37	3.77	10.7	13.1
38	3.88	11.0	13.5
39	3.98	11.3	13.8
40	4.08	11.6	14.2
41	4.18	11.9	14.5
42	4.28	12.2	14.9
43	4.39	12.5	15.2
44	4.49	12.8	15.6
45	4.59	13.0	15.9
46	4.69	13.3	16.3
47	4.79	13.6	16.6
48	4.90	13.9	17.0
49	5.00	14.2	17.4
50	5.10	14.5	17.7
51	5.20	14.8	18.0
52	5.30	15.1	18.4
53	5.40	15.4	18.7
54	5.51	15.7	19.0

These ranges are somewhat more narrow than those obtained if the hemoglobin coefficient and five million erythrocytes per cu. mm. are used in the color index formula.[36] The smaller range gives a margin of safety to eliminate borderline cases having slight abnormal erythrocyte morphology, where the linear correlation of the microhematocrit to the

erythrocyte count and hemoglobin value is not valid.

If a hemoglobin value for a given microhematocrit reading falls outside these calculated values, the latter procedure is repeated on a new sample of blood. An erythrocyte count is done if the same discrepancy is noted.

The question arises as to the limitations to be placed on the use of the linear correlation of the microhematocrit to the erythrocyte count and the hemoglobin content. Freedman[11] limited his use of the correlation to the range of 40 to 52 hematocrit units. The lack of a sufficient number of samples in the range below 35 in our results makes it unwise to assume at this time that a linear correlation continues to exist below a reading of 35 hematocrit units. Experience with the method, however, shows that the lower part of the line could possibly be used where normal erythrocyte morphology is established. Further work is being done on this phase at the present time. The adaptation of the lower end of the line would further increase the usefulness of the method in a busy laboratory.

The amount of data available to fix the upper limit is also scant. For the present time the relationships are not used above 54 hematocrit units.

The linear correlation between the microhematocrit and the erythrocyte count has not been shown to be valid in children under two years of age. Their erythrocytes normally show marked anisocytosis with a tendency toward macrocytosis. Consequently, the microhematocrit is not routinely used to replace the erythrocyte counts for patients in this age group.

In accordance with the work of Price-Jones,[23, 31] the presence of hemorrhage causes a decrease in cell size amounting to 0.9 microns from the normal mean size of 7.21 microns. This would tend to prohibit the use of the microhematocrit on patients suffering from massive hemorrhage.

In a study of thirty patients suffering from hemorrhage no apparent gross erythrocyte morphology indicative of a decrease in the average cell size was noted upon examination of stained smears. The microhematocrit procedure was used in conjunction with the erythrocyte count and hemoglobin estimation to follow the course of the bleeding and subsequent treatment by blood transfusion. It was found that the erythrocyte count varied widely while the microhematocrit and hemoglobin estimation closely followed the bleeding and treatment. Figures 4, 5 and 6 are representative of our findings.

This is further evidence that the microhematocrit gives a more satisfactory indication of the state of the erythrocyte content of the blood in the absence of abnormal erythrocyte morphology than does the single periodic erythrocyte count ordinarily used.

The microhematocrit has been substituted for the erythrocyte count in all routine and admission complete blood counts at this laboratory and has been in use for over a year. The method has been found very satisfactory. The number of erythrocyte counts has been reduced from approximately 120 per day to between ten and fifteen. The daily number of erythrocyte counts may be broken down into three groups, five to

eight of these counts are from children under two years of age, one to four are for platelet counts by the Dameshek method[35] and the remainder are microhematocrits with hemoglobin values falling outside the range given in Table 6 and those specifically requested by physicians.

In these days of minimal technologist staffs in clinical laboratories the lack of accuracy can frequently be traced to insufficient time available to complete large daily work loads. Since the blood sample can be taken from the patient and the microhematocrit prepared, centrifuged and read by an adequately trained technician assistant, many man hours are saved for the medical technologist. This leaves additional time for the medical technologist to concentrate on the more complex and difficult procedures.

Having established the feasibility of using the microhematocrit as an effective substitute for the erythrocyte count in routine blood counts, it was decided to establish a normal range for this test and for the erythrocyte count, hemoglobin, erythrocyte indices and constants.

Reference to Table 5 will show that the erythrocyte count, microhematocrit and hemoglobin normals obtained by us are considerably lower than those suggested by Wintrobe.[33, 37] Values close to ours were found by Walters[32] in his study of 100 healthy males in Kansas. The series of 3,000 soldiers examined by Freedman[11] in Louisiana has shown an even lower set of values. These observations add further weight to the opinion that each locality must prepare its own set of normal ranges, and in order directly to compare the results obtained with those of others in different localities, a coefficient should be used.

The hemoglobin and volume coefficients are suggested by Osgood[20] as one method of universal comparison. The value for the hemoglobin coefficient obtained in this study was found to be 15.6 for males and 15.5 for females. Thus, using this coefficient and five million erythrocytes per cu. mm. as 100 per cent, the normal color index for this area for both males and females is 1.00. The volume coefficient was found to be 48 for males and 48.8 for females. The volume index is, therefore, 1.00 for both sexes. Consequently, the saturation index is also 1.00.

These indices are in accordance with those generally accepted as normal.

The mean corpuscular volume, hemoglobin and hemoglobin concentration given in Table 5, have been calculated from the microhematocrit results. McGovern[19] suggested the direct prediction of the Wintrobe hematocrit level from the result of the microhematocrit on venous blood by using the formula

$$c = 2.1 \text{ plus } 0.98b$$

where b = the microhematocrit level and c = the Wintrobe hematocrit level. Thus, for the normal male microhematocrit given in Table 5, the Wintrobe hematocrit would be 47 and the normal female Wintrobe hematocrit would be 42. These are identical with Wintrobe's normal packed cell volumes.[33] The mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration from the calculated Wintrobe packed cell volumes would be 100 cu. microns, 31.88 micromicrograms and 32 per cent respectively.

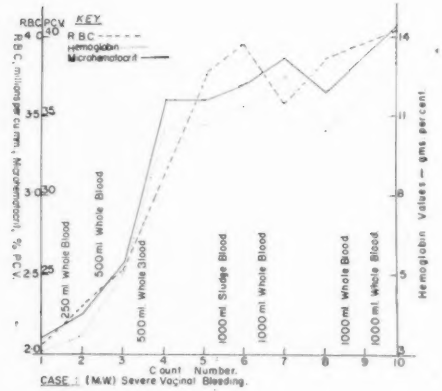


Figure 4
Microhematocrit v. Erythrocyte Count.

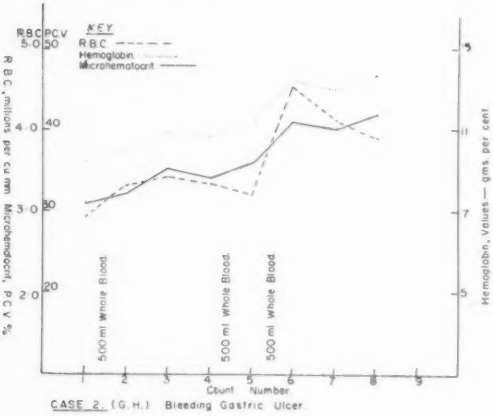


Figure 5
Microhematocrit v. Erythrocyte Count.

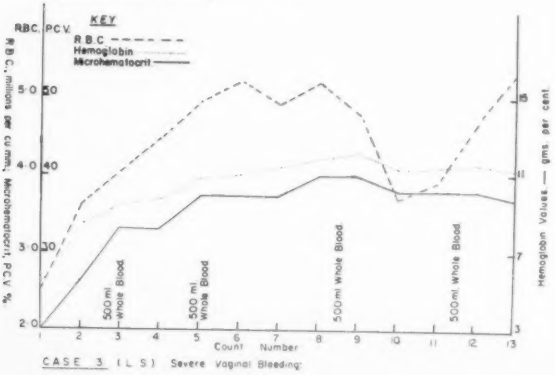


Figure 6
Microhematocrit v. Erythrocyte Count.

Conclusions

The microhematocrit is simple, saves time and is accurate. Reproducibility far exceeds that of the erythrocyte count.

In the range of 35 to 54 units there is a close linear correlation between the microhematocrit, the erythrocyte count and the hemoglobin content in blood samples having normal erythrocyte morphology.

The substitution of the microhematocrit for the erythrocyte count is practical.

The combination of microhematocrit and hemoglobin gives a much better picture of the state of the erythrocyte content of the blood sample in single routine examinations than does the erythrocyte count and hemoglobin value.

The normal values of males and females for this section of the United States are lower than those of Wintrobe. The values, the indices and constants are similar to those found by Walters for 100 males in the State of Kansas.

Each laboratory wishing to adopt this procedure as a routine should set up regression lines for themselves. At the same time, normal values for the area could be calculated. If this were done by two or three widely separated laboratories in each State and the results pooled at a central clearing house, some indication of the distribution of these important hematologic values would perhaps be given and a better estimate of the nationwide normal ranges made.

Summary

1. An evaluation of the erythrocyte count, microhematocrit and hemoglobin for this laboratory was undertaken.

2. The evaluation showed that the microhematocrit is the most reproducible test of the three, the erythrocyte count the least reproducible.

3. The use of a small column of blood in the microhematocrit is necessary to avoid gross variations in packed cell volume due to an uneven bore in 75 mm. commercial capillary tubes.

4. A regression line was constructed for the microhematocrit and erythrocyte count and for the microhematocrit and hemoglobin to show that a linear correlation exists between these two pairs of tests.

5. A table of hemoglobin values for the purpose of checking the microhematocrit was prepared.

6. Normal values for male and female erythrocyte counts, microhematocrits and hemoglobins, together with indices and constants are given for this part of the United States.

REFERENCES

1. Armstrong, W. D., Singer, L., and Dunshee, B. R.: Measurements of Radio Isotopes in Blood, Applied to the Determination of True Hematocrits. Proc. Soc. Exp. Biol. and Med., 80: 639-642, 1952.
2. Beams, J. W., and Weed, A. J.: Scientific Apparatus and Laboratory Methods. A Simple Ultra-Centrifuge. Science, 74: 44-46, 1931.
3. Berkson, J., Magath, T. B., and Hurn, M.: The Error of Estimate of the Blood Cell Count as Made with the Hemocytometer. Am. J. Physiol., 128: 309-323, 1939-1940.
4. Biggs, R., and MacMillan, R. L.: The Errors of Some Hematological Methods as they are used in a Routine Laboratory. J. Clin. Path., 1: 267-287, 1948.

5. Biggs, R., and MacMillan, R. L.: The Error of the Red Cell Count, J. Clin. Path., 1: 288-291, 1948.
6. Blum, L. L.: Photometric Determination of an Erythrocyte Count; Basic Principles and Technic. Am. J. Clin. Path., 15: (Tech. Supp. 9) 85-93, 1945.
7. Bönniger. M.: Die Bestimmung des Blütkorperchenvolumens. Berl. Klin. Wchnschr., 46: 161, 1909.
8. Brecher, G., Schneiderman, M., and Williams, G. Z.: Evaluation of an Electronic Blood Cell Counter. Am. J. Clin. Path., 26: 1439-1449, Dec. 1956.
9. Drabkin, D. L.: Standardization of Hemoglobin Measurement. Am. J. Med. Sc., 215: 110, Jan. 1948.
10. Drabkin, D. L.: Standardization of Hemoglobin Measurement. Am. J. Med. Sc., 217: 710, June, 1949.
11. Freedman, A. M., and Mirsky, A. J.: Estimation of Red Cell Count from Hematocrit. Am. J. Clin. Path., 16: 104-109, 1946.
12. Guest, G. M., and Siler, V. E.: A Centrifuge Method for the Determination of the Volume of Cells in Blood. J. Lab. Clin. Med., 19: 757-768, 1933-1934.
13. Hald, A.: Statistical Theory with Engineering Applications. N. Y., Wiley and Sons, 1952, pp. 522-557.
14. Hepler, O. E.: Manual of Clinical Laboratory Methods. 4th Ed., Springfield, Ill., Charles C. Thomas, 1956, p. 356.
15. Kendrick, A.: The Application of Centrifugal Force. International Equipment Company, Information Bulletin No. 1, 1952, pp. 2-3.
16. Jones, A. R.: A Device for Rapidly Deriving the Hematocrit of Blood Centrifuged in Ungraduated Tubes. New Eng. J. Med., 254: 172-174, Jan., 1956.
17. Leverton, R. M., and Roberts, L. J.: Hemoglobin and Red Cell Content of the Blood of Normal Women during Successive Menstrual Cycles. J.A.M.A., 106: 1459-1463, 1936.
18. Magath, T. B., Berkson, J., and Hurn, M.: The Error of the Determination of the Erythrocyte Count. Am. J. Clin. Path., 6: 568-579, 1936.
19. McGovern, J. J., Jones, A. R., and Steinberg, A. G.: The Hematocrit of Capillary Blood. New Eng. J. Med., 253: 308-312, 1955.
20. Osgood, E. E.: Hemoglobin, Color Index, Saturation Index, Volume Index Standards; redeterminations Based on Findings in 137 Healthy Young Men. Arch. Int. Med., 37: 685-705, 1926.
21. Parker, G. M., Spicer, H. E., and Porter, H.: A Method for Computing the Red Cell Count on the Photoelectric Colorimeter. Am. J. Clin. Path., 14: (Tech. Supp. 8) 37, 1944.
22. Parpart, A. K., Ballentine, R.: Hematocrit Determination of Relative Cell Volume. Science 98: 545-546, 1943.
23. Price-Jones, Cecil: Red Blood Cell Diameters. Oxford University Press, American Branch, New York, 1933.
24. Shils, M. E., Sass, M., and Goldwater, L. J.: A Microhematocrit Method and Its Evaluation. Am. J. Clin. Path., 22: 155-159, 1952.
25. Shohl, A. T.: The Measurement of Cell Volume of Blood by the Cell Opacity Method. J. Lab. Clin. Med., 25: 1325-1332, 1940.
26. Shohl, A. T., Blackfan, K. D., and Diamond, L. K.: Cell Opacity Method for Determination of Cell Volume on a Single Drop of Rat's Blood. Proc. Soc. Exp. Biol. and Med., 45: 383-387, 1940.
27. Smirk, F. H.: I. The Accurate Measurement of the Proportion of Corpuscles and Serum in Blood. II. The Volume of Red Blood Cells in Venous and Oxygenated Blood after Exposure to Various Saturations of CO_2. Brit. J. Exper. Path., 9: 81, 1921.
28. Smith, C.: Daily Erythrocyte Counts in Menstrual and Intermenstrual Periods. Am. J. Physiol., 114: 452-456, 1936.
29. Strumia, M. M., and Principato, L. A.: The Air Turbine Hematocrit for Measurement of the Relative Volume of Packed Cells. Am. J. Clin. Path., 20: 419-428, May 1950.
30. Strumia, M. M., Sample, A. B., and Hart, E. D.: An Improved Microhematocrit Method. Am. J. Clin. Path., 24: 1016-1024, 1954.
31. Vaughan, W. T.: Erythrocyte Diameters. J. Lab. Clin. Med., 19: 335-338, 1933-1934.
32. Walters, O. S.: Normal Erythrocyte, Hemoglobin and Packed Cell Volume Standards in Young Men. J. Lab. Clin. Med., 19: 851-864, 1933-1934.

33. Wintrobe, M. M.: Clinical Hematology, 4th Ed., Philadelphia, Pa., Lea and Febiger, 1956, p. 100.
34. Ibid: p. 102.
35. Ibid: p. 267.
36. Ibid: pp. 392-394.
37. Wintrobe, M. M., and Miller, M. W.: Normal Blood Determinations in the South. Arch. Int. Med., 43: 96-113, 1929.

AMONG THE NEW BOOKS

CLINICAL CHEMISTRY—PRINCIPLES AND PROCEDURES. by Joseph S. Annino, Clinical Chemist, Massachusetts Memorial Hospitals, Boston. Little, Brown & Company—Boston. 1956. 280 pp. 15 illustrations, 11 tables, 2 charts, $7.50.

As the author aptly describes it, this book is written on the "technician level" and "is intended to fill the gap between the 'cook-books'" . . . "and the theoretical text books." It does that to a large extent. By pre-supposing a knowledge of most of the fundamentals of quantitative analysis as well as biochemistry, Part I touches briefly upon *Basic Technics and Fundamental Information*, through the chapters on "Laboratory Apparatus and Standard Solutions", "Colorimetry," and "A Review of Quantitative Analysis".

Part II, on *Methods*, goes into recommending methods and techniques. Featured here is the adaptation of technique to more than one of the body fluids. For example, a determination may be described to test urine and blood, or blood, spinal fluid and urine, or to whole blood or serum. Much of the contents of this volume may be found in others, but one would undoubtedly search through *more* pages to find what he wants.

HEMORRHAGIC DISEASES. by Armand J. Quick, Ph.D., M.D., Professor of Biochemistry, Marquette University School of Medicine, Milwaukee, Wisconsin. Lea & Febiger, Philadelphia, Penna. 1957. 451 pp. Illustrated. 31 Tables. $9.50.

While Part I, describing the chemical aspects of the Hemorrhagic Diseases, is of great interest to the medical technologist, the second portion of this book on Laboratory Methods is of top ranking value. The important, and often neglected, coagulation and bleeding times, platelet counts, clot retraction and tourniquet tests are given complete coverage through brief notes on principle, equipment and reagents, and details on procedure, application and interpretation. In addition the author includes the recent advances on newly introduced clotting factors: the labile and stable factors, anti-thromboplastinogen, its detection and determination, and the preparation and assay of thromboplastinogen. No hospital clinical laboratory should be without this volume.

A TEXTBOOK OF HISTOLOGY. Seventh Edition by Alexander A. Maximow, late Professor of Anatomy, University of Chicago, and William Bloom, Professor of Anatomy, University of Chicago, Illinois. W. B. Saunders Company, Philadelphia, Penna. & London, England. 1957. 628 pages. 1082 illustrations, 265 in color, 631 figures. $——.

With the increasing interrelation of chemistry and histology, new techniques have been developed. With the solution of some of the problems in histology, others have arisen. Many new illustrations have been added to this section. Electron Micrographs have increased the value of this book immensely, and the color illustrations are as beautiful as any we have seen. While it is a "must" in the Histology laboratory, this volume is of considerable worth in any clinical laboratory.

LABORATORY GLASS BLOWING. by L. M. Pan, M. Inst. B.E. in collaboration with C. A. Hendley Chemical Publishing Co., Inc., New York, New York. 1957. 154 pp. many illustrations and diagrams. $4.00.

As glass blowing for the average clinical technical worker is fast becoming a "lost art", this book will provide some practical guidance to an interesting facet of the laboratory. Both simple and more complex apparatus-making are described. The illustrations and instructions will enable the unskilled glass blower to develop an ability to handle glass, if for no other reason than to make simple repairs.

MEDICAL MANUAL OF CHEMICAL WARFARE. SYMPOSIUM. Fourth Ed. Chairman of Editorial Committee, Major-General A. Sacks, C.B.E., M.Sc., M.D., M.R.C.P., Q.H.P. War Office. Chemical Publishing Company, Inc., New York, New York. 1956. $4.00.

On general principles, this volume should be on the shelves of all hospital libraries—not necessarily in the laboratory. There is little practical application for the medical technologist except that each of us should have a "reading" knowledge of the subject, at least. The book is written for quick reference, and primarily for the physician.

A NEW COLORIMETRIC TEST FOR ALBUMINURIA

By A. S. GIORDANO,* M.D., NORA ALLEN,* B.S., M.T. (ASCP), MARTHA WINSTEAD,* A.B., M.T. (ASCP), and MARY ALICE PAYTON,** M.T. (ASCP)
*South Bend Medical Foundation, South Bend, Indiana; and
**Sarasota Memorial Hospital, Sarasota, Florida

Introduction

A test for albumin is an important and informative aspect of any urine analysis regardless of whether such tests are carried out in a hospital laboratory, in a physician's office, or as a part of some multiphasic screening program. With the commonly used sulfosalicylic acid tests, turbid urines and variable lighting can cause difficulties in determining whether or not a small amount of albumin is present. The problem of turbid urines can be minimized by centrifuging. Some of these urines remain turbid even after centrifuging and, therefore, still present a problem.

This paper describes the experiences of the authors with Albutest† a new colorimetric tablet test for the detection of albuminuria. Results obtained have been compared with a sulfosalicylic acid procedure. The data were obtained in three different laboratories by the authors and by technologists working under them. Tests were carried out by typical and usual hospital laboratory personnel.

Methods

Exton's sulfosalicylic acid test has been used routinely by our laboratories in testing urines for albumin. The test is applied to a portion of the supernatant urine obtained after centrifuging. Exton's reagent[1] contains 20 per cent sodium sulfate and 5 per cent sulfosalicylic acid. The actual test is carried out by combining approximately equal amounts of centrifuged urine and Exton's reagent.

The mixture is warmed gently over an open flame and then read immediately to avoid the increased turbidity which develops on standing. The varying degrees of turbidity were described as follows by two of the laboratories:

	Lab I	Lab II
negative	no turbidity	no turbidity
trace	somewhat cloudy	somewhat cloudy
1+	very cloudy, no granules	definite cloud
2+	fine granules	heavy cloud, no granules
3+	flocculation	granular (300 mg. = 3+)
4+	separates to curds	separates to curds

Amounts of turbidity which some people report as "faint trace" are reported as "negative" in our laboratories.

Albutest, the colorimetric tablet test, was used according to the directions supplied by the manufacturer. These directions were as follows:

1. Place tablet on clean surface and put one drop of urine on tablet.
2. After urine has been absorbed, add two drops of water and allow to penetrate before reading.

† Albutest is a registered trademark of Ames Company, Inc., Elkhart, Indiana.

3. Compare color of tablet with color photograph. The color photograph shows typical reactions obtained with a negative urine, a urine containing a small amount of albumin, and a urine containing a large amount of albumin.

The tablet contains an indicator and a buffer. In the absence of albumin the indicator is dominated by the buffer and has a light yellow color. In the presence of albumin, the color of the indicator changes to produce a light bluish green spot with a small amount of albumin and a dark blue spot with a large amount of albumin.

Results

The results obtained by the three laboratories have been tabulated separately in Table I. A total of 2228 urines were tested by the three laboratories. Laboratory No. 1 tested 780 urines, laboratory No. 2 tested 1215 urines with actual values being given for 278 while the remaining 937 urines were reported only that results with both albumin tests agreed, and laboratory No. 3 tested a total of 233 urines. All three laboratories found that comparable results were obtained with both the sulfosalicylic acid test and the colorimetric albumin test.

TABLE I

Results Obtained With the Sulfosalicylic Acid and the Colorimetric Tests for Albuminuria

Sulfosalicyclic Acid Test	COLORIMETRIC TABLET TEST					Total Urines Tested
	Neg.	Trace	1+	2+	3+	
Laboratory No. 1:						
Neg.	578	6	..	..	..	
Trace	31	74	8	..	..	
1+	..	6	26	5	..	
2+	2	..	8	19	4	780
3+	..	..	..	3	10	
4+	..	..	..	..	..	
Laboratory No. 2:						
Neg.	189	2	..	..	..	
Trace	7	31	7	..	..	
1+	..	2	12	4	4	
2+	..	..	..	5	3	278
						937*
3+	..	..	1	..	9	
4+	..	..	..	..	2	1215
Laboratory No. 3:						
Neg.	142	2	3	..	..	
Trace	12	5	17	..	..	
1+	..	4	9	4	4	
2+	..	..	1	8	4	233
3+	..	..	..	1	8	
4+	..	..	..	..	9	

*Not tabulated since results with parallel tests were reported only as agreeing.

Bence-Jones protein gave a positive reaction with the colorimetric tablet test. This was demonstrated with a urine already recognized as containing Bence-Jones protein. The urine was acidified, heated to boiling, and filtered while hot. The filtrate gave a positive reaction with the tablet test.

A thorough study was made of a urine from a patient who had been given Telepaque as a gallbladder X-ray contrast media. This urine sample was negative with the tablet test but gave 1+ Exton reaction, 3+ nitric acid ring test, and 1+ heat and acid test. The rate of precipitation with sulfosalicylic acid was much slower than is typical of albumin.

The addition of acetic acid alone, without preliminary heating of the urine, also caused a precipitate to form. The material contained in the urine was clearly demonstrated to be nonprotein. It readily dialyzed through a membrane, gave a negative Biuret test, and was unaffected by proteolytic enzymes. The material which was readily precipitated with acid could be easily redissolved by addition of alkali. It has previously been recognized[2] that several X-ray contrast media may result in so-called false positive albumin tests. The colorimetric tablet test is not affected by X-ray contrast media and gives a correctly negative test in the absence of albumin.

It was established that a urine sample from a patient receiving pyridium contained albumin. The reaction was originally interpreted as positive although the presence of pyridium gave an off-color to the reaction. Similar results were observed when albumin was added to urine which contained pyridium. In the absence of albumin, pyridium caused no interference and negative reactions were readily apparent.

One particularly outstanding feature of the tablet test was its ready application to turbid urines. Turbid urines could be tested as easily as clear ones. In our laboratories turbid urines cause very little interference with sulfosalicylic acid precipitation tests since urines are routinely centrifuged and albumin tests are run on the supernatant. Occasionally, however, a urine does not clear completely with centrifuging. In one laboratory a record was made of the number of turbid urines and it was found that 12 per cent of the urines received in the laboratory were turbid.

The shortage of technologists automatically places a premium value on any modification or new test which gives accurate and reliable results but requires less of the technologist's time for its performance. The tablet test requires considerably less time than other albumin tests. This savings in time results partially from the simplicity of the test itself and partially from its completely disposable nature. Technicians ran albumin tests on ten urines at a time using a different method for each group of ten urines. The time required was divided by ten to give the average time to run one test. The average times obtained were as follows: over 1 minute for a heat and acetic test, over ½ minute for a nitric acid ring test or a sulfosalicylic acid test, but less than ¼ minute for the colorimetric tablet test.

Discussion

Results obtained with the colorimetric tablet test appear to correlate quite well with Exton's test as used in our laboratories. While results reported with Exton's in each of our three laboratories correlate quite well, it is appreciated that these tests may be read differently in different laboratories. The colorimetric tablet test includes a color photograph to show typical reactions and, therefore, facilitates standardization of readings and reports. The specificity of the tablet test is good. In a few instances a positive reaction was obtained with only one of the two tests. With the colorimetric tablet test, turbid urines can be tested without preliminary treatment.

Summary

Parallel studies were carried out in three separate laboratories. Albutest, a new colorimetric tablet test for the detection of albuminuria, was compared with a sulfosalicylic acid procedure. Good agreement was found with the two methods. Results with the tablet test apparently were not affected by various drugs or metabolities which might be present in random urines from hospital patients. Properties of the tablet test provide basis for its practical usefulness in hospital laboratory practice.

ADDENDUM

Since submitting this paper for publication a new form of the colorimetric test has been developed. The new test is called Albustix[2] and is a dip-test employing the same principles as Albutest. The active ingredients in this case have been impregnated into a paper stick. After dipping the stick into urine, the color of the stick is compared with the color chart to obtain the milligrams of protein present per 100 ml. of urine. The time required to run the colorimetric stick test is even less than that required for the colorimetric tablet test. A series of over 1000 urines has been run in which Albustix is compared with Albutest and with other protein tests. The stick test, like the tablet test, is specific for protein and does not give false reactions with drug metabolites.

REFERENCES

1. Todd, J. C., Sanford, A. H., and Wells, B. B.: *Clinical Diagnosis by Laboratory Methods*, 12th edition, 1953, p. 77, W. B. Saunders Co.
2. McMillin, T. J.: Pseudo Proteinuria Due to Radiopaque Substances, Northwest Medicine **55:** 301-302, March 1956.

[2] Albustix is a trademark of Ames Company Inc., Elkhart, Indiana.

ADDENDA TO A REPORT OF C-REACTIVE PROTEIN*

BERYL MARIAN DIMMICK, MT (ASCP)
Pittsburgh Branch Laboratory, Penna. Dept. of Health, Pittsburgh, Pennsylvania

Introduction

C-Reactive Protein (CRP) has been described as an "acute phase reactant" which appears in human serum in response to a variety of inflammatory stimuli. Believed to be an alpha globulin it appears rapidly, within 14 to 24 hours after inflammation or tissue injury has occurred. This abnormal globulin was reported in 1930 by Tillet and Francis,[1] who noted its presence in the acute phase of pneumococcal pneumonia and its disappearance during convalescence. The protein was isolated and identified by MacLeod and Avery,[2] and the name C-Reactive was derived from its capacity to be precipitated by the somatic C-polysaccharide of the pneumococcus. A specific antiserum sensitive enough to detect minute amounts of CRP (concentrations of less than 0.01 mg. per cc.) has been produced by hyperimunization of rabbits with repeated injections of purified C-Reactive Protein.

The usual criteria for necrosis and inflammation include fever, leucocytosis and an elevated erythrocyte sedimentation rate. Investigators have claimed that the C-Reactive Protein determination is most valuable in clinical situations where these criteria are absent or equivocal. Although the test is not specific for any single inflammatory disease or any groups of inflammatory conditions most investigators seem to agree that it is the most sensitive indicator of an inflammatory process available in our present laboratory routines. A number of factors may influence the fall of red cells in the sedimentation rate, varying with the individual and in different diseases. The C-Reactive Protein, on the other hand, appears in the blood as a single factor in response to the inflammatory process. Since there are only positive and negative readings no "normal" range of values complicates interpretation of results.

C-Reactive Protein has been reported in various diseases by numerous investigators. It has been demonstrated in conditions characterized by inflammatory reaction such as rheumatic fever, pneumococcal pneumonia, staphylococcal osteomyelitis, subacute bacterial endocarditis,[1,3,4,7] in lupus erythematosus, in the majority of cases of rheumatoid arthritis,[8] and in myocardial infarction.[9] Investigators in evaluating possible serological tests for cancer have reported positive CRP levels in a large majority of patients with well defined cancer.[10] Anderson and McCarty have reported that changes in amount of C-Reactive Protein tend roughly to parallel changes in the erythrocyte sedimentation rate, but the CRP may be absent from the blood when the sedimentation rate is higher than normal and may be present when the sedimentation rate has returned to normal.[6] Hedlund found that no parallel exists between the white blood count and the presence of the "acute phase protein" (CRP).[5] All reports agree that C-Reactive Protein is the most consistently positive laboratory test in the presence of rheumatic activity.[6]

* Third Award—Scientific Products Foundation—Serology Division. Read before the Annual Convention, Chicago, Illinois, June 1957.

Procedure

The test is an easily read antigen-antibody reaction in which a white precipitate is formed in a clear solution.

We have followed the technique provided with the antiserum prepared by Schieffelin and Company, New York, using 0.4 mm. x 90 mm. capillary tubes and two hour incubation followed by overnight refrigeration at 6 to 8° C. Although the tests included in this report were not set up as a controlled experiment the specimens were all treated in relatively the same manner. This should be mentioned since some recent investigators have reported variations in CRP results after prolonged storage of serum—at room temperature—in the refrigerator—and in the deep freeze.[12] The protein has been accused of being "ubiquitous" in nature, and the test has been labeled unstable as well as nonspecific. The majority of our bloods were fasting specimens drawn in the morning. Most of the C-Reactive Protein determinations were performed within a maximum of forty-eight hours after collection. All sera* used had been inactivated at 56° C. for 30 minutes.**

Approximately half of the sera were separated from the clot within four hours after collection. The other half remained on the clot for 24 to 48 hours before separation and inactivation. Samples were stored at room temperature for no longer than six hours and at 6 to 8° C. for the remaining time before testing. Known positive and negative controls were included with each series of determinations.

Results

A preliminary report, submitted one year ago, was based upon the first one hundred C-Reactive Protein determinations performed in our laboratory over a period of six months. Results of these tests were correlated with the clinical diagnosis, the erythrocyte sedimentation rate, the white blood count and the Anti-Streptolysin O titer. We have continued our observations in order to compare the results of a much larger group of C-Reactive Protein determinations with the preliminary figures. A total of 814 determinations (representing 699 Individual patients) has been performed over a period of eighteen months. Of these approximately 60 percent have been positive and 40 percent negative.

As shown in Table 1, our group is a broadly representative one composed of male and female, white and negro, ranging from 14 to 78 years of age.

TABLE 1

Comparison of C-Reactive Protein With Sex and Color

	Percent Positive CRP	Percent Negative CRP	Percent of Total Group Surveyed
White	65%	35%	84%
Negro	80%	20%	16%
Male	65%	35%	45%
Female	54%	46%	55%

* Patient's serum must be perfectly clear before performing the test. Plasma from oxalated or citrated blood is unsatisfactory.[6]

** While the directions do not mention inactivating patient's serum we routinely use serum inactivated at 56° C. for 30 minutes since AST-O are requested on many of the same specimens. Parallel CRP determinations on inactivated and non inactivated serum in our laboratory produced similar readings. The protein will, however, be destroyed by heating above 65° C.[4]

The negro group appeared to indicate a higher ratio of positive to negative CRP than did the white. It should be noted, however, that the group under consideration was considerably smaller in size than the group of white patients.

All of the determinations have been done on hospitalized patients for whom C-Reactive Protein levels were specifically requested since the test is not a routine admission procedure in our hospital. The original group of one hundred determinations previously reported is included in all figures presented. As mentioned in our former report a control group was included which was composed of one hundred non patient individuals from whom we expected negative C-Reactive Protein readings. This group included premaritals, pre-employment, food handlers and students.

Table 2 shows the diagnoses represented and the results of the C-Reactive Protein determinations. The categories must, of necessity, be quite broad, and, in general represent those most frequently discussed in the literature.

TABLE 2

DIAGNOSIS	No. Patients	Percent Positive CRP†	Percent Negative CRP
Rheum. Fever and RHD	171	53%	47%
Rheum. Arthritis	41	70%	30%
Non Rheum. H. D.	192	67%	33%
Infections*	110	58%	42%
Cancer	19	70%	30%
Collagen Disease, Osteo-Arthritis, Lupus	42	60%	40%
Miscellaneous‡	124	50%	50%
Control Group	100	10%	90%

† CRP—C-Reactive Protein.

Note: Those reactions showing a trace of CRP have been included in the positives in the preparation of all tables, since we are told that even a trace of CRP is abnormal.

* Infections listed above include: Pneumonitis, bronchitis, glomerulo, sinusitis, hepatitis, cholecystitis, diverticulitis and pancreatitis.

‡ Miscellaneous group includes: Diabetes, various surgicalp rocedures, infectious mononucleosis and types of anemia.

Previous investigators have reported a rough parallel between the ESR and CRP.[5] The figures given in Table 3 are based upon a single erythrocyte sedimentation rate and single C-Reactive Protein performed at approximately the same time. The Wintrobe method was used for the ESR determination with normal range of 0 to 9 mm. for males and 0 to 20 mm. for females.

TABLE 3

Comparison of C-Reactive Protein and Erythrocyte Sedimentation Rate

ESR* and CRP†	Per cent of Group Surveyed
Elevated ESR and Positive CRP	52%
Elevated ESR and Negative CRP	22%
Normal ESR and Positive CRP	6%
Normal ESR and Negative CRP	20%

* ESR—Erythrocyte Sedimentation Rate.
† CRP—C-Reactive Protein.

Hedlund and others have reported little or no correlation between the C-Reactive Protein and the white blood count. 10,000 WBC per cmm.

was used as an arbitrary limit of normal range for the comparison shown in Table 4.

TABLE 4

Comparison of C-Reactive Protein and White Blood Count

	Percent of Group Surveyed
WBC* less than 10,000 and Positive CRP†	39%
WBC higher than 10,000 and Positive CRP	21%
WBC less than 10,000 and Negative CRP	34%
WBC higher than 10,000 and Negative CRP	6%

*WBC—White Blood Count.
†CRP—C-Reactive Protein.

Most investigators have found no correlation between the C-Reactive Protein and Anti-Streptolysin-O titer. The figures below are based upon a single Anti-Streptolysin-O titer and single C-Reactive Protein performed on the same sample of serum and at approximately the same time. 224 units has been used arbitrarily as an equivocal significant value for a single Anti-Streptolysin-O determination in the preparation of Table 5.

TABLE 5

Comparison of C-Reactive Protein and Anti-Streptolysin-O Titer

	Percent of Group Surveyed
Positive CRP* and ASTM-O† within normal range	50%
Negative CRP and AST-O within normal range	32%
Positive CRP and AST-O 224 units or higher	8%
Negative CRP and AST-O 224 units or higher	10%

* C-Reactive Protein.
† Anti-Streptolysin-O titer.

The highest temperature recorded for the patient in the 24-hour period preceding collection of the specimen was used in the preparation of the following table.

TABLE 6

Comparison of C-Reactive Protein and Temperature

	Percent of Group Surveyed
Temperature 98.6 to 99.6° and Negative CRP*	39%
Temperature 98.6 to 99.6° and Positive CRP	49%
Temperature higher than 99.6° and Negative CRP	2%
Temperature higher than 99.6° and Positive CRP	10%

*CRP—C-Reactive Protein.

Summary

A survey has been made of 814 C-Reactive Protein determinations performed over a period of 18 months upon a widely representative group of hospitalized patients. These were not routine admission procedures but were specifically requested. Results of the C-Reactive Protein test have been correlated with the clinical diagnosis, the erythrocyte sedimentation rate, the white blood count, the Anti-Streptolysin O Titer and the patient's temperature. A control group of 100 non patient individuals was included in the preparation of the comparisons. Our survey of a large group shows basically the same distribution as that shown in our

preliminary report on a much smaller group one year ago. The larger group showed positive C-Reactive Protein reactions in approximately 60% while the smaller group showed approximately 85% positive. The sedimentation rate and C-Reactive Protein correlated well, as reported previously, with the greatest degree of correlation appearing in the elevated sedimentation rate and positive CRP group. Our findings in the comparison of the white blood count and C-Reactive Protein agree with those of Hedlund and others who report little or no correlation. We found better correlation between the negative CRP and normal white blood count than between the positive C-Reactive Protein and increased white count. As was to be expected our results reveal no correlation between the C-Reactive Protein and the Anti-Streptolysin O Titer. Poor correlation was demonstrated too between the C-Reactive Protein results and temperature readings with C-Reactive Protein appearing earlier than the elevation in temperature. Thus, our figures would indicate that the C-Reactive Protein is a nonspecific but highly sensitive test for inflammatory processes. In many instances it appears earlier than or in the absence of the accepted indicators of inflammation—the elevated erythrocyte sedimentation rate, the increased white blood count and the elevated temperature. Our findings in this larger group agree with those presented in a brief preliminary report one year ago and continue to agree with reports previously published.

BIBLIOGRAPHY

1. Tillet, W. S. and Francis, R., Jr.: Serological Reactions in Pneumonia with a Non-Protein Somatic Reaction of Pneumococcus. J. Exper. Med. 52:561-571, October, 1930.
2. MacLeod, C. M., and Avery, O. T.: The Occurrence During Acute Infections of a Protein not Normally Present in the Blood. Isolation and Properties of the Reactive Protein. J. Exper. Med. 73:183, February, 1941.
3. Bunim, J. J., Kittner, A. G., Baldwin, J. S. and McEwen, C.: Cortisone and Corticotropin in Rheumatic Fever and Juvenile Rheumatoid Arthritis. J. A. M. A. 150:1273, November, 1952.
4. Stollerman, G. H., Glick, S., Patel, D. J. Hirschfeld, I., and Rusoff, J. H.: Determination of CRP in Serum as a Guide to the Treatment and Management of Rheumatic Fever, Amer. J. Med. 15:645, November, 1956.
5. McEwen, C., and Ziff, M.: Basic Sciences in Relation to Rheumatic Diseases. M. Clin. North America, 39:765, May, 1953.
6. Anderson, H. C., and McCarty, M.: Determination of CRP in the Blood as a Measure of the Activity of the Disease Process in Acute Rheumatic Fever. Amer. J. Med. 8:445, April, 1950.
7. Wood, H. F., and McCarty, M.: Laboratory Aids in the Diagnosis of Rheumatic Fever and in the Evaluation of Disease Activity. Amer. J. Med. 17:768, December, 1954.
8. Hill, A. G. S.: C-Reactive Protein in the Chronic Rheumatic Diseases. Lancet. 2:807, November, 1951.
9. Hedlund, P.: The Appearance of Acute Phase Protein in Various Diseases. Acta Med. Scandinav. Supplement, 196:579, 1947.
10. Sprunt, D. H., and Hale, W. M.: Proceedings American Association Cancer Research. Volume 2, April, 1955.
11. Havens, W. P. Jr., Eichman, H. L., and Knowlton, M.: Failure to Find C-Reactive Protein in Viral Hepatitis. Proc. Soc. Exper. Biol. and Med. 75:108, October, 1950.
12. Knights, E. M., Hutchins, M., Morgan, E., and Ploompuu, J.: Use of the C-Reactive Protein Test as a Screening Procedure of Blood Donors. Journal AMA, Volume 162, No. 1:9, 1 September, 1956.

AN ANALYSIS OF THE CRITERIA USED TO DETERMINE THE PATHOGENESIS OF STAPHYLOCOCCI INCLUDING A COMPARISON OF THE SLIDE AND THE TUBE COAGULASE TEST*

MRS. DOROTHY L. NESS, MT (ASCP)
913 Thornton Street, S. E., Minneapolis 14, Minnesota

Loeb,[14] in 1903, recognized that certain staphylococci had the ability to coagulate plasma. The production of staphylocoagulase is generally considered the best single test for the estimation of the pathogenicity of a given strain.[6,8] For clinical purposes this test is of relative value, for occasionally coagulase-negative staphylococci will invade tissue and cause suppuration. However, with rare exception, severe cases of staphylococcal sepsis have been associated with coagulase-positive staphylococci.[16] There are two methods to test for the presence of staphylocoagulase. The slide method was first observed by Much[15] in 1908 and confirmed by Birch-Hirschfeld[1] in 1934. The tube method, first reported by Loeb in 1903, has since been studied by numerous workers and is generally accepted as the standard by which the slide method may be evaluated. The slide method detects coagulase which is bound to the cell wall. Bound coagulase acts directly on the fibrinogen of certain animals which then causes clumping of the staphylococcal cells.[10] Free coagulase, detected by the tube test, is liberated into the medium. It is the precursor of a thrombin-like substance, the production of which depends upon the participation of an activator present in some plasmas but deficient or lacking in others.[19] Bound coagulase, or "clumping factor," is antigenically distinct from free coagulase.[10] Most workers agree that pathogenic staphylococci usually possess both free and bound coagulase.[4] Other species of microorganisms were tested. Positive tube tests[8,11] and positive slide tests[4] were both found to be specific for staphylococci.

One hundred strains of staphylococci, consisting of both coagulase-positive and -negative types, isolated consecutively during a one-month period, were studied. All strains had been subcultured once to trypticase soy agar slants.

Table I reviews the sources of the 100 strains. The purpose of this study is threefold: I. To compare the slide and tube method for detecting staphylocoagulase. II. To compare the results in the tube test using fresh human plasma (diluted and undiluted), lyophilized human plasma, and fresh or desiccated rabbit plasma. III. To compare manitol fermentation, chromogenesis, and hemolytic activity with the coagulase test.

PART I

Methods

The slide method, as used in this laboratory, is done as follows:

1. A small drop of sterile distilled water† is placed on a slide. Four cultures are usually tested on the same slide.

* First Award—Scientific Products Foundation—Bacteriology Division. Read before the Annual Convention, Chicago, Illinois, June, 1957.

† Dropper bottles with small tips such as used for blood typing sera are sterilized and used for this purpose.

2. A heavy, smooth suspension of staphylococci is made with an inoculating loop from the original isolation plate or from an agar slant subculture if there was not enough growth on the original plate.

TABLE I

Sources and Coagulase Reactions of 100 Strains of Staphylococci Isolated Consecutively from 12-15-56 Through 1-15-57

SOURCE	NUMBERS OF STRAINS	
	Coagulase Positive	Coagulase Negative
Abscess or wound	27	9
Blood	14	6
Throat	7	2
Nose	4	3
Sputum	5	0
Urine	5	2
Vagina	1	2
Abdominal fluid	1	0
Eye	2	1
Ear	2	0
Feces	1	4
Cerebrospinal fluid	0	1
Totals	69	31

3. A drop of undiluted fresh human plasma* is added and mixed with the loop. Very distinct clumping should occur within 5-15 seconds to be coagulase-positive. Delayed reactions, representing non-specific agglutination and smooth suspensions are considered coagulase-negative.

4. The slides are allowed to dry and are stained with Gram's stain to confirm that the organism is a Gram-positive coccus.

The colony characteristics of young yeast and some diphtheroids may be confused with staphylococci. For this reason, Gram's stain is recommended. Gram's stain is also helpful in that the coagulase-negative staphylococci are usually large sized cells, whereas the coagulase-positive staphylococci are usually smaller in size. Diphtheroids are quite often immiscible. Three per cent of the original isolates of staphylococci were immiscible and therefore unsuitable for the slide test. Subcultures of these organisms gave smooth suspensions which could then be tested. It should be noted that the most frequent cause of false negative slide tests is an insufficiently heavy suspension of organisms.

The tube method, as used in this laboratory, is carried out on all blood cultures as follows:

1. A suspension of cells from the original plate or subcultured slant is made with an inoculating loop directly in 0.5 ml. of fresh human undiluted pasma. One-half ml. of the original blood culture broth is sometimes used as inoculum to save time.

2. Incubate at 37° C. and observe at 1, 2, 3 and 4 hours for a clot. A known coagulase-positive control is included.

Results

Table II shows a comparison of the slide and tube methods. There is a failure of 3 of the 100 strains to agree in the two tests. These 3 strains are tube-positive and slide-negative. No slide-positive, tube-negative strains were seen. The slide test confirmed 95.7 per cent of the coagulase-positive tube tests.

Human plasma diluted 1:4 in sterile distilled water was also used in doing slide coagulase tests on the 100 organisms of this series. No advantage was seen in using diluted plasma for the slide test. Four per cent of the staphylococci clumped faster in undiluted plasma than in diluted plasma. Ninety-six per cent showed no difference in speed of reaction.

TABLE II

Comparative Results of Tube and Slide Methods for Detecting Presence or Absence of Staphylocoagulase in 100 Strains of Staphylococci

NO. OF STRAINS	Tube Method	Slide Method
62	+	+
4	sl +	+
2	sl +	—
1	+	—
31	—	—
0	—	+

Discussion

Cadness-Graves, Williams and Miles[4] in 1943 reported agreement of the tube and slide methods for detecting staphylocoagulase in all but 2 of 442 strains studied by one experienced worker. In a study of 860 strains by 3 workers, the slide test confirmed 90 per cent of the strains positive by the orthodox tube method. There were no false positive slide tests.

Needham, Ferris, and Spink[16] in 1945 reported a failure of the tube and slide methods to correlate in only 7 of 513 strains. The 7 exceptions were tube-negative and slide-positive. They emphasized the fact that this work was done by one experienced worker. Their results did not show a single strain that was tube-positive and slide-negative. When the results from 1313 strains tested by students were compiled, there was a correlation of approximately 95 per cent between the two tests. In this series there were 38 tube-positive, slide-negative tests, and 27 tube-negative, slide-positive tests.

The differences noted in the data of one worker, compared with two or more suggest that the slide test should be adopted only after a preliminary trial by each observer of the two methods in parallel.[4] The stage of experience necessary to do an accurate slide test is easily reached. The proper use of the slide test may reduce the number of tube tests by more than 50 per cent.

A satisfactory interpretation of the results in 2 per cent of the organisms in our series still remains to be resolved by more data. These strains, which are slide-negative, form a slight clot in the tube, only after 24 hours incubation. They are mannitol-negative, non-chromogenic, and non-hemolytic. Both of the strains were judged by the physician to be of doubtful significance in these specific cases. Chapman[6] states that any amount of clot at 24 hours should be called coagulase-positive. Many workers agree with this, since no correlation exists between the degree of coagulation and the pathogenicity of the organism.

One strain in our series produced a positive tube, but a negative slide test. It was isolated from an ear, was hemolytic, mannitol-positive, but white in color. It was evaluated by the physician to be clinically significant. Strains which are only tube positive are found occasionally in the

nose and throat and are of doubtful pathogenicity.[10] As a consequence it would seem that the slide test alone may be an adequate test for pathogenicity. At present we feel it is necessary to do tube tests on all slide negatives.

A plasma agar plate technique for determining coagulase activity has been proposed by Reid and Jackson.[18] Blair[2] has questioned the specificity of this method and suggests further investigation. Clapper and Wood[7] compared human plasma plates with the tube coagulase test using human and rabbit plasma.

PART II

According to Duncan and Walker[9] the result of the tube coagulase test is influenced by the proportion of culture to plasma in the mixture. The optimal proportion can be determined by titrating increasing dilutions of plasma against a fixed volume of culture. The antigenicity of coagulase is very low, though specific antibody was produced by Rammelkamp, *et al.*[17] by the injection of rhesus monkeys. Ninety three per cent of 307 adult human plasmas studied by Tager and Hales[20] showed evidence of resistance to coagulase, ranging from complete refractoriness to varying degrees of inhibition. Most workers[8,5] agree that diluted human plasma is preferable to undiluted human plasma. Cadness-Graves states that diluting plasma in broth rather than saline for direct inoculation of the colony into the test medium is far more sensitive. Data recently collected in this laboratory shows that broth is far superior to water as a diluent for plasma. Rabbit plasma, according to Chapman,[3] gives a faster clot, but the final results are similar regardless of the speed of clotting. He also states that rabbit plasma clots progressively less firmly so it should be used as fresh as possible. Human plasma kept at 0-2° C. for 6 weeks was still satisfactory.[11] Kaplan and Spink[12] conclude that the difference in susceptibility of various plasmas to coagulase may be a quantitative phenomenon which is dependent upon the relative amounts of coagulase, activator of coagulase and inhibitor of coagulase. They found that dried or frozen human plasma had less activator substance than fresh human plasma. Rabbit plasma was deficient in inhibitor substance.

Methods

The human plasma used in the previous study was titrated against a fixed volume of culture. The optimal dilution, in which a clot appeared most quickly was 1:4. Tube tests were done on the 100 strains using 1:4 diluted plasma to compare with undiluted plasma. In both cases the tubes were inoculated directly from agar slants and incubated for three hours in a 37° C. water bath and then allowed to stand at room temperature overnight.

For the purpose of evaluating one organism in desiccated rabbit plasma, fresh rabbit plasma, lyophilized human plasma, or fresh human plasma, a saline suspension was made. Two-tenths ml. of this was added to 0.5 ml. of the various kinds of plasma in order to obtain a more uniform inoculum. The fresh rabbit plasma, when titrated against a fixed volume of culture, clotted fastest in a 1:4 dilution. All plasmas tested were diluted 1:4 in sterile water.

Results

An analysis of Table III, which compares human plasma diluted 1:4 with undiluted human plasma, reveals that 11 per cent of the strains produced a clot in the diluted plasma one hour sooner than in the undiluted plasma.

TABLE III

Comparative Results of Tube Method for Detecting Presence or Absence of Staphylocoagulase in 100 Strains of Staphylococci Using Fresh Human Plasma Undiluted and Diluted 1:4

No. STRAINS		Hours of Incubation At 37° C.			Room Temp.
		1	2	3	24
8	Undiluted	—	+	+	+
	Diluted	+	+	+	+
1	Undiluted	—	—	—	+
	Diluted	—	—	sl.	+
2	Undiluted	—	+	+	—
	Diluted	+	+	+	—
19	Undiluted	sl.	+	+	+
	Diluted	+	+	+	+
1	Undiluted	—	sl.	+	+
	Diluted	—	+	+	+
3	Undiluted	sl.	+	+	—
	Diluted	+	+	+	—
6	Undiluted	—	—	—	—
	Diluted	sl.	sl.	sl.	sl.
1	Undiluted	sl.	sl.	sl.	sl.
	Diluted	—	—	—	—
23	Undiluted	+	+	+	+
	Diluted	+	+	+	+
31	Undiluted	—	—	—	—
	Diluted	—	—	—	—
3	Undiluted	sl.	+	+	+
	Diluted	sl.	+	+	+
2	Undiluted	+	+	+	—
	Diluted	+	+	+	—

In 23 per cent, there was a good clot in diluted plasma when only a slight clot was present in the undiluted plasma. Six per cent of the undiluted had no clots even after 24 hours but had slight clots in the diluted. This gives a total of 40 per cent in which the diluted plasma gave the better reaction. One per cent produced a slight clot in undiluted plasma when no clot was present in diluted plasma. Fifty-nine per cent showed no difference in length of time to obtain a clot.

It is also shown that of the staphylococci which formed a clot after 1 or 2 hours at 37° C., seven per cent lysed after incubation at room temperature for 24 hours. This represents the action of a fibrinolysin-like

substance. The organisms appeared to produce, not a true fibrinolysin, but an activator, staphylokinase, of profibrinolysin.[13]

Sixty-seven out of 69 coagulase-positive staphylococci clotted plasma to some degree within one hour's incubation in a 37° C. water bath. If a 37° C. air incubator is used, the clotting is often delayed one or two hours depending on how often the door is opened.

A commercially available human lyophilized plasma was compared with the fresh citrated human plasma obtained from the blood bank. On a limited study of only 11 strains of staphylococci, 9 coagulase-positive and 2 coagulase-negative, the results were very similar. A clot was obtained faster with fresh plasma in 3 strains. Boyd,[3] in a study of 8 strains, reported identical results using the lyophilized or fresh human plasma. The choice of which kind of human plasma to use would depend upon the most convenient source of supply.

Fresh citrated rabbit plasma and a commercially available desiccated rabbit plasma were compared with human plasma on 19 strains, 17 coagulase-positive and 2 coagulase-negative. In this limited study, again, very little difference was seen in the length of time required for the clot to form. A firmer clot was obtained with rabbit plasma than with human plasma in 5 strains.

PART III

Various biological tests have been used as an indication of the pathogenicity of a particular staphylococcal strain. Toxin formation, as evidenced by rabbit cell hemolysis, destruction of leukocytes, or necrosis in the skin of a rabbit, is not suitable for general application in routine work.[11]

Lactose fermentation and gelatin liquefaction are used by some investigators in attempting to separate pathogens from non-pathogens.

The criteria of pathogenicity most often used in clinical laboratories are hemolytic activity, mannitol fermentation, chromogenesis and coagulase.

Methods

Hemolytic activity was noted on 5 per cent sheep blood agar incubated overnight at 37° C. Moderate or pronounced hemolysis was considered positive. Very slight hemolysis was considered negative. One per cent mannitol fermentation tubes with brom-cresol purple indicator were observed for seven days. Chromogenesis was determined by the ability of the organisms to produce gold, yellow or white pigment on Loeffler's medium. The slants were incubated at 37° C. for 18 hours and then were subsequently left at room temperature for another 24 hours. This procedure, according to Fairbrother[11] enhances the pigment production.

Results

Table IV shows the comparative results of these tests performed on the same 100 strains. There was a correlation between hemolysis and staphylo-coagulase production in only 69 per cent of the isolates. It may be noted that a large number, 29 per cent of the staphylococci were not hemolytic but coagulase-positive, whereas only 2 per cent were hemolytic but coagulase-negative.

Eleven strains fermented mannitol (acid without gas) in one day, 51 strains in two days, 3 strains in three days, and 6 strains in more than three days. Twenty-nine strains failed to ferment mannitol in 7 days. The fermentation of mannitol in 7 days and the production of staphylo-

coagulase is directly correlated in 88 per cent of the strains studied. Chromogenesis showed the best direct correlation with staphylocoagulase production, *viz.*, 91 per cent. All strains in this series were gold (aureus) or white (albus). No yellow pigmented strains (citreus) were encountered.

TABLE IV

Comparative Results of Mannitol Fermentation, Chromogenesis, and Hemolysis With the Presence or Absence of Staphylocoagulase in 100 Strains of Staphylococci

Staphylocoagulase		Mannitol			Hemolysis	Chromogenesis
			3 Days*	7 Days*		
+	69	+	63	64	+40	+62
		—	6	5	—29	— 7
—	31	+	2	7	+ 2	+ 2
		—	29	24	—29	—29

*Length of incubation.

Discussion

The use of such terms as "hemolytic staphylococcus aureus" based on information obtained by blood agar plate is condemmed by Fairbrother. He states that satisfactory evidence has never been produced showing this type of hemolysis to be the same as soluble hemolysin, an index of toxin formation. Fairbrother noted that when mannitol is fermented by pathogenic strains, it is accomplished almost invariably in 3 days. Saprophytic strains, in contrast, ferment only after 3 days. On the basis of this, the direct correlation of mannitol fermentation in 3 days and the production of coagulase would be 92 per cent. Cruickshank emphasizes the difference between two albus varients, distinguished by hemolysis and coagulase production. In our series, 29 out of 36 albus strains were coagulase-negative, and 33 out of 36 albus strains were non-hemolytic.

On the basis of the data obtained from this study and the data obtained by other workers, our laboratory will now adopt the following policy for the detection of the presence or absence of staphylocoagulase:

1. Slide coagulase tests using undiluted fresh human plasma will be performed on most staphylococci isolated. Unless they are the predominant organism, we do not do coagulase tests on staphylococci from the nose, throat, sputum, urine or feces.

2. Tube coagulase tests, using fresh human plasma diluted 1:4 in tryptose phosphate broth, will be performed only on slide-negative, immiscible, or late-clumping staphylococci. Tubes will be incubated in a 37° C. water bath for three hours. If they are negative at this time, they will stand overnight at room temperature and be reported on the next day.

Summary

The rapid slide mehod for the detection of staphylocoagulase has been compared with the orthodox tube method. The two methods were found to be in agreement in 97 of the 100 staphylococcal strains tested. The slide coagulase test constitutes a simple, time saving, reliable method that is recommended for diagnostic bacteriology. The tube coagulase test should be performed on all slide-negative staphylococci. A comparison of fresh human undiluted plasma, fresh human diluted plasma, fresh

rabbit plasma, lyophilized human plasma, and desiccated rabbit plasma for use in the tube test is discussed. In this laboratory the fresh human diluted plasma has been found to be the most satisfactory. Various criteria for the separation of pathogenic from saprophytic staphylococci have been compared with the production of staphylocoagulase. There is a better agreement between the coagulase reaction and chromogenesis than there is between coagulase reaction and mannitol fermentation. Hemolytic activity is unreliable and can apparently not be correlated with the other three criteria.

BIBLIOGRAPHY

1. Birch-Hirschfeld, L., Über die Agglutinatiön Von Staphylokokken durch Bestandteile des Säugetierblutplasmas, Klin. Wschr., **13**:331, March 3, 1934.
2. Blair, J. E., Dubos, René J., *Bacterial and Mycotic Infections of Man.* Second edition, Philadelphia, Pennsylvania, J. P. Lippincott Company, 1952.
3. Boyd, H., An Evaluation of Lyophilized Plasma in the Coagulase Tests for Micrococcal Pathogenicity, Am. J. Med. Tech., **22**:232, July-Aug., 1956.
4. Cadness-Graves, B., Williams, R., Harper, G. J., and Miles, A. A., Slide-test for Coagulase-positive Staphylococci, *Lancet*, **1**:736, June 12, 1943.
5. Chapman, G. H., Berens, C., and Stiles, M. H., The Coagulation of Plasma by Staphylococci, J. Bact., **41**:431, Aug., 1940.
6. Chapman, G. H., Berens, C., Nilson, E. L., and Curcio, L., The Differentiation of Pathogenic Staphylococci from Non-pathogenic Types, J. Bact., **35**:311, March, 1938.
7. Clapper, W. E., Wood, D. C., Comparison of Three Methods for the Determination of Coagulase Activity of Staphylococci, J. Bact., **67**:545, May, 1954.
8. Cruickshank, R., Staphylocoagulase, J. Path. & Bact., **45**:295, July, 1937.
9. Duncan, J. T. and Walker, J., Staphylococcus aureus in the Milk of Nursing Mothers and the Alimentary Canal of their Infants, J. Hyg., **42**:474, Oct., 1942.
10. Duthie, E. S., Evidence for Two Forms of Staphylococcal Coagulase, J. of Gen. Mic., **10**:427, June, 1954.
11. Fairbrother, R. W., Coagulase Production as a Criterion for the Classification of the Staphylococci, J. Path. and Bact., **50**:83, Jan., 1940.
12. Kaplan, M., Spink, W., Studies of the Staphylocoagulase Reaction: Nature and Properties of a Plasma Activator and Inhibitor, Blood, **3**:573, May, 1948.
13. Lack, C. H., Staphylokinase: An Activator of Plasma Protease, Nature, London, **161**:559, April 10, 1948.
14. Loeb, L., The Influence of Certain Bacteria on the Coagulation of the Blood, J. Med. Research, **10**:407, Oct., 1903.
15. Much, H., Über eine Vorstufe des Fibrinfermentes in Kulturen von Staphylokokkus aureus, Biochem. Ztschr., **14**:143, Sept., 1908.
16. Needham, G. M., Ferris, V., Spink, W., The Correlation of the Rapid Slide Method with the Tube Method for Differentiating Coagulase-positive from Coagulase-negative Strains of Staphylococci, Am. J. Cl. Path., **15**:83, Nov., 1945.
17. Rammelkamp, C. H. Jr., Badger, G. F., Dingle, J. H., Feller, A. E., Hodges, R. G., Antigenicity of Cell-free Staphylococcal Coagulase, J. Inf. Dis., **86**:159, Mar.-Apr., 1950.
18. Reid, J. D., and Jackson, R. M., An Improved Method for Determining the Coagulase Activity of Staphylococci by Means of the Plasma Agar Plate, J. Lab. & Clin. Med., **30**:155, Feb. 1945.
19. Smith, W., Hale, J. H., The Nature and Mode of Action of Staphylococcus Coagulase, Brit. J. of Exper. Path., **25**:101, June, 1944.
20. Tager, M., Hales, H. B., Differences in the Resistance of Human Plasmas to Staphylocoagulase, Yale J. Biol. & Med., **21**:91, Oct., 1948.

THE EFFICIENCY AND ECONOMY OF ROUTINE MICRO-METHODS*

LESLIE W. LEE

Laboratory Service, Lake View Hospital, Danville, Illinois.

Anyone who has worked in laboratories for over ten years is aware of the constantly increasing load laboratories are carrying. In one decade many of us have seen our work load doubled and more with the increase of routine tests and the introduction of new technics unheard of a few short years ago. New technologists are being trained and old ones are being kept abreast of the times by study courses, journals and conferences such as this one. But the training is not able to keep pace with the constantly accelerating demand for trained medical laboratory personnel. If we are to fulfill our mission of providing prompt, adequate and reliable laboratory service we must somehow solve this problem.

As I see it, either we must recruit, train and place an enormous number of qualified technologists, we must train aides who can relieve us from some of the more time-consuming and less meticulous chores, or we must be prepared to turn out many more tests per day ourselves. All three of these methods are, of course, being explored and used. Recruiting and training well qualified personnel has occupied the attention of this group for many years but there is reason to doubt that this solution alone will provide adequate help to do all of our work at current rates of productivity, and there is some doubt as to the economic feasibility of this solution. Training of aides has also relieved the shortage but there is serious question of the wisdom of relying too heavily on partially trained help.

I believe it is obvious that the most fruitful course for us and for our profession lies in the direction of increasing the amount of work we can individually turn out in a given period of time. We have threatened, begged and cajoled the manufacturers into putting much equipment onto the market to save us time and increase our efficiency but it seems to me we have not gone as far as we might in increasing our effectiveness by simple modifications of methods and stream-lining of work loads. This approach takes time to test our changes against accepted methods and time is a rare commodity indeed in a busy laboratory.

During the past five years we, in our laboratory, have been studying this problem and have arrived at some solutions which we feel have been noteworthy. Many of the ideas are not new but we feel that we have combined many of these ideas into an effective program and have proven their usefulness, reliability and economy.

While I will cite figures, in many cases, to testify to the reliability of these methods, I am sure someone will question their absolute accuracy. While I am a strong advocate of accuracy, I feel that the utility of a method must be determined by its clinical reliability and during the past five years we have proven this quality for these methods to our satisfaction.

---

*Third Registry Award. Read before the Annual Convention, Chicago, Illinois, 1957.

On the question of accuracy, I would like to make a few points about micro-methods in general.

1. In pipetting, the hold-back is extremely variable between solutions because of their surface tension. Thus a 1 ml. pipette will deliver quite different quantities of sulfuric acid and ether. With a micro-pipette calibrated "to contain" 0.1 ml. the solution is rinsed from the pipette with the diluent and all of the 0.1 ml. of any solution is actually delivered.

2. Glassware manufacturers have provided us with micro-glassware calibrated as accurately as the comparable routine item.

3. If you do red and white blood cell counts you are doing micro-technics and you should have no difficulty mastering the few simple rules of routine micro-chemistry.

4. The same chemical reactions take place in 5 or 50 ml. of solution. There is nothing fundamentally different about these methods and the technics do not get intricate until the ultra-micro range is reached.

5. Standards, blanks and recoveries must be run just as in macro-methods, particularly until a modification is proven.

Let me say at this point that our aim has been the evolution of easier, faster and more economical methods with no particular interest in the quantities involved in the reactions. Quantities of reagents and sample have been kept in the range where they are most easily handled.

Let us take for example two methods of performing a total protein using the Phenol method of Folin and Ciocalteu. The technic we were using five years ago went as follows: Transfer 1 ml. of serum to a test tube containing 9 ml. of .85 sodium chloride. Mix and transfer 2 ml. of this serum dilution to a 50 ml. volumetric flask. Add 25 ml. of saline and 2 ml. of 5N sodium hydroxide. Mix and add 3 ml. of Phenol Reagent. Dilute to volume, mix, let stand for 10 minutes and read.

Using the same reagents in the same proportions we arrive at the following technic. Place 4.5 ml. distilled water in a cuvette. Wash in 0.02 ml. serum. Add 0.2 ml. of 5N sodium hydroxide, mix and add 0.3 ml. of Phenol reagent. Mix, let stand 10 minutes and read.

Let us assume that there are one-thousand total proteins performed by the two technics and project our supply, dish washing, technician time, etc. on this basis. By method one we will probably have used 1,000 50 ml. volumetric flasks, 1,000 chemical test tubes, 6,000 large pipettes, 1,000 cuvettes, about 60 liters of saline, sixteen four-ounce bottles of sodium hydroxide and twenty-four four-ounce bottles of Phenol Reagent. This is 9,000 pieces of glassware and 65 liters of fluid. This would nearly fill a small truck. In method number two we have used three thousand micropipettes, 1,000 large pipettes and 1,000 cuvettes. About five liters of distilled water would have been used and an eight ounce bottle of each of the reagents. These items would go in a large suitcase.

We routinely keep a small bottle of these reagents in a rack on the counter top while the test is being done as I will explain later. Compare the distance from your hand to such a rack with the distance from your hand to your nearest reagent shelf. Multiply each by 1,000 tests and again by 3 reagents and you will get an idea of the needless time and effort expended in a year on one test due solely to the positioning of the reagents. By the first of these methods the patients would have lost

about ten units of blood compared to about 50 ml. by method number two.

As to the accuracy and reproducibility of this method, one standard deviation in our laboratory was 0.22 grams compared to 0.26 grams for the routine method. Since the average deviation from one method to the other was only .13 gms. of which there was an average .13 minus deviation and a .07 plus deviation, it was felt that the curve prepared for the standard method was sufficiently accurate for clinical work. Routine standards and recoveries showed accuracy to be very good.

Another specific method which we have found to be of value is our modification of the BUN method of Karr. One ml. filtrate is placed in the bottom of a 5 ml. volumetric flask. One-tenth ml. of Urease-Glycerol and .05 ml. of buffer are added. Flasks are incubated for 15 minutes in a 50° C. water-bath. The flask is then half-filled with distilled water, a drop of gum ghatti is added and one c. c. of Nessler's reagent is blown in. Volume is adjusted to 5.0 ml. and the reading is made. Several systems were tried but this one appeared to work the best. A separate curve was prepared. One standard deviation for the method was 1.24 mg compared to 2.73 for the macro-method. Daily standards show consistent, reliable and clinically acceptable accuracy. It should be mentioned, however, that in this particular method, dirty glassware and careless technic will show up quickly.

A modification of the Zak cholesterol method has also proven itself in our laboratory. In this case 0.1 ml. of serum is blown into about 3 ml. of acetone-alcohol solution in a graduated centrifuge tube. The tube is immersed in a boiling water bath for about a half minute, then cooled and the volume adjusted to 5.0 ml. with acetone-alcohol. The tube is stoppered and centrifuged and 2.5 ml. of the centrifugate is transfered to a chemical test tube where it is evaporated to dryness. Three ml. of glacial acetic acid and 2.0 ml. of the color reagent are added to the warm tube. The contents are mixed and allowed to cool. Color can be read on the curve prepared for the standard method. Standards and recoveries for this modification show excellent accuracy and reproducibility.

A modification which we are currently studying is the NPN using one ml. of filtrate. The filtrate is placed in the bottom of a 10 ml. volumetric flask with 0.2 ml. of 50% H_2SO_4 and two drops of hydrogen peroxide. The flask is placed on a thremostatic hot plate at about 225° C. Within about ten minutes the digestion will be completed as evidenced by the absence of new fumes arising from the filtrate. After time and temperature are established this step can be timed. The flasks are cooled and half-filled with water. Two drops of gum ghatti are added and 3.0 ml. of Nessler's solution is blown in. The volume is adjusted and the contents mixed and read. Accuracy studies have not been completed as this is being written but standards have been quite accurate and the method shows much promise. By this method a dozen NPN's are run as easily as two or three by the conventional method. Reagents and blood are saved and the normally bothersome fumes are reduced in quantity and escape only slightly from the long necks of the flasks. The digestion is accomplished at a lower temperature which avoids spattering and heavy boiling which carry off nitrogen and give a lower result.

Nearly all methods in our chemistry department have been modified

and studied in this way. Several changes have been made in the arrangement and operation of the department with the idea of efficiency in mind. Tungstic acid deproteinization of blood is done by centrifugation in all cases using 0.5 ml. of blood or less. Samples are obtained by venipuncture wherever it is convenient since we are interested primarily in saving time.

I have been impressed by the amount of time lost in walking to the refrigerator, the centrifuge, the hood, the glassware cabinets, etc. All of these facilities can be arranged within reach of a seated technologist if micro-methods are used. The commonly used reagents that must be refrigerated are stored in a single rack and set out at the time pipetting of reagents is started each morning. Other reagents are arranged directly in front of the technologist. Since the reagents may be stored in small bottles, all of the reagents normally used may be kept within arm's reach and the technician may remain seated during almost the entire working interval. A table-top centrifuge is adequate in most cases. The glassware consists almost entirely of test tubes, 5 and 10 ml. flasks and small pipettes which may be stored in drawers at either side of the technologist. In our own case the colorimeter is also in the area of reach.

The flame photometer, the CO_2 apparatus and such large pieces of equipment are not too adaptable to the "reach area." Many people, however, have found that a reduced quantity of serum dilution was more convenient for the flame instrument. For CO_2 combining power determinations we have used the Kopp-Matelson microgasometer which uses 0.03 ml. of serum and have found it to be faster and more economical of space, serum, and mercury than the Van Slyke apparatus.

Summary

By devising micro-modifications of routine methods we have been able to use smaller quantities of reagents and blood, smaller and fewer pieces of glassware and much less time in performing routine chemical methods. The consequent reduction in storage and work space has enabled us to save additional time previously taken in walking, reaching for large bottles and sorting through a crowded refrigerator. Technologists who have worked with this system have found it very easy to learn and to teach and have been enthusiastic about it. The saving in time has amounted to almost 40% and the saving in supplies equals or exceeds that amount. I believe studies such as these may go a long way toward allowing us to do the work we have to do in the time we have to do it.

BIBLIOGRAPHY

1. Zak, B. et al: "Rapid Estimation of Free and Total Cholesterol," Am. J. Clin. Path., 24: 1307-1315, 1954.
2. Natelson, S.: "Routine Use of Ultra-Micro Methods in the Clinical Laboratory," Tech. Bull., 21: 125, 1951.
3. Henry, J. H., Berkman, S., Golub, O. J., and Segalove, M.: "Analysis of Error in Clinical Chemical Determinations and Pipetting Procedures," Tech. Bull., 23: 21-30, 1953.
4. Folin, O. and Ciocalteu, V.: "On Tyrosine and Tryptophane Determinations in Proteins," J. Biol. Chem., 73: 627, 1927.
5. Saller, Sister Mary William: "A Companison of Total Serum Protein and Albumin Values as Determined by Micro-Kjeldahl, Biuret and Folin Methods," Am. Jour. Med. Techn., 21: 1-15, 1955.
6. Karr, W. G., "A Method for the Determination of Blood Urea Nitrogen," J. Lab. & Clin. Med., 9: 329-333, 1924.
7. Connerty, H. V., Briggs, A. R. and Eaton, E. H., "Determination of Blood Urea Nitrogen Using a Simple Stabilizing Reagent," Tech. Bull., 25: 247-251, 1955.

AN A-B HEMOLYSIN SCREENING TEST AS A ROUTINE BLOOD BANK PROCEDURE*

EDITH L. BOSSOM, A.B., M.T. (ASCP) BB
Blood Bank, University of Kansas Medical Center, Kansas City, Kansas

Although there is an increasing awareness of the importance of transfusing group-specific blood, all transfusion services must meet situations which require the use of heterospecific blood. The dangers inherent in the indiscriminate use of group O have been emphasized by numerous reports of hemolytic transfusion reactions.[2,7,8] There is general agreement that some sort of anti-A and anti-B screening procedure should be performed in order that these heterospecific transfusions will not be unnecessarily hazardous. The question of which screening technic to follow in the selection of "safe" donors is still a matter of debate and investigation among blood bank workers.

The choice of screening method would be much less difficult if it were known what elements of group O blood render it dangerous for universal use. It may be high titers of naturally-occurring agglutinins, elevated titers of "immune" agglutinins, potent hemolysins, a combination of these antibodies, or other components not yet discovered. A review of the literature indicates that hemolysins and "immune" isoagglutinins are regularly found in "universal donor blood" which has caused hemolytic reactions. It is not surprising, therefore, that attention is now being focused on screening tests designed to detect "immune" and hemolytic antibodies.

Among the various characteristics of the "immune" antibody is its ability to sensitize red cells to an anti-human globulin serum after neutralization of the normally-occurring isoagglutinins with group specific substance. Grove-Rasmussen *et al.*[10,11] have developed an indirect Coombs screening test based on this property. Another attribute of the "immune" agglutinin is its ability to hemolyze A or B red cells in the presence of complement. Screening tests based on this hemolytic property have been suggested by Gardner and Tovey,[9] Dunsford and Bowley,[6] and further investigated by workers in this country.[3,12]

This paper is concerned with: (1) a comparison of the hemolysin screening test with the screening procedure involving neutralization of isoagglutinins with group specific substance followed by the indirect Coombs test, (2) a comparison of the hemolysin test with the 1:200 saline isoagglutinin method, (3) a comparison of the hemolysis resulting from the use of 2 per cent and 5 per cent suspensions of red cells in the hemolysin test, and (4) experience with the hemolysin screening method as a routine test in a university hospital blood bank.

Materials and Methods

Donor specimens. Unoxalated specimens of donor blood are allowed to clot at room temperature, after which they are centrifuged to obtain clear serum. Hemolysin tests are usually performed on sera within six hours after collection. Alternately the sera are stored at 4 to 6° C. for no more than 24 hours before testing. A fresh group O serum free of hemolysins

* ASMT Award—Read before the Annual Convention, Chicago, Illinois, June, 1957.

is the source of complement for sera unavoidably more than 24 hours old.

Suspensions of A and B cells. Saline suspensions of cells are prepared daily from specimens of clotted blood not more than three days old.[3] Cells from a single group B blood are used and a pool of A cells is prepared from three group A bloods. The cells are pipetted into each of two 15 ml. centrifuge tubes and are washed four times with fresh changes of 0.85 per cent saline solution. After final centrifugation, readings of the packed cell volumes are taken and the cells are diluted to accurate 5 per cent concentrations. The pooled A cells are tested with absorbed anti-A serum and are used in the hemolysin test only if they are strongly reacting A_1 cells. A portion of each 5 per cent suspension is transferred to a second set of test tubes and diluted with saline to an approximate 2 per cent concentration for use in the serum confirmation typings (reverse groupings), which are routinely performed in the processing of donor bloods. The 2 per cent suspensions were also used in the comparison series of screening tests whenever the indirect Coombs or 1:200 saline isoagglutinin tests were carried out.

Test for normally-occurring isoagglutinins. The 1:200 saline dilution test for normally-occurring isoagglutinins is not performed as a part of our routine procedures but was carried out on 77 group O, 84 group A, and 38 group B sera for comparison with results of the hemolysin screening method.

A 1:200 dilution of serum was prepared by pipetting 10 ml. of 0.85 per cent saline into a test tube containing exactly 0.05 ml. of the donor serum being tested. Thorough mixing was accomplished by inverting the diluted serum several times on a clean stopper. By means of capillary pipets, equal volumes (3 drops) of the 1:200 dilution of serum and a 2 per cent suspension of A_1 cells were pipetted into tube "A"; equal parts of the diluted serum and a 2 per cent suspension of B cells were placed in tube "B". The tubes were left at room temperature for one hour after which they were centrifuged for two minutes at 1000 r.p.m. Agglutination was graded from negative to 4+ by macroscopic and microscopic examination but for purposes of comparison with the results of the hemolysin tests, recordings were divided into the two categories of positive and negative.

Test for "immune" isoagglutinins. The indirect Coombs tests for "immune" anti-A and anti-B are not performed routinely but were carried out on a series of 150 group O, 133 group A, and 51 group B sera for comparison with the results of the hemolysin screening procedure.

One-tenth milliliter of donor serum was pipetted into a test tube containing 0.3 ml. of combined A and B group specific substance.* The mixture was shaken and allowed to neutralize for one hour at room temperature. By means of capillary pipets, equal volumes (3 drops) of the neutralized serum and a 2 per cent suspension of A_1 cells were added to tube "A"; equal parts of neutralized serum and a 2 per cent suspension of B cells were placed in tube "B". The tubes were shaken gently and incubated in the 37° C. water bath for one hour, after which the Coombs test was performed on each tube as follows: (1) The tests were washed

* Combined A and B group specific substances may be obtained from Sharp and Dohme, Philadelphia, Pa. Separate A and B group specific substances are available from Knickerbocker Biosales, Inc., New York, N. Y.

three times with fresh changes of saline. (2) After the last washing all saline was decanted and 2 drops of Coombs reagent added to each tube. (3) The packed cells were resuspended in the Coombs serum by gentle shaking and the tests were centrifuged for two minutes at 1000 r.p.m. Macroscopic readings were made. Negative or doubtful agglutination constituted a negative result. Clear-cut agglutination was recorded as a positive result.

Sera titered for "immune" antibody content were first neutralized in a 1 to 4 dilution with group specific substance as just described for the qualitative test. Serial, doubling dilutions of the neutralized serum were made in saline. Three drops of the appropriate 2 per cent suspension of A_1 or B cells were added to each tube, the titer was incubated in the 37° C. water bath for two hours, and the Coombs test was carried out on each tube. Readings were determined by macroscopic examination.

Test for hemolytic isoagglutinins. The hemolysin screening tests have been incorporated into the routine processing procedures and are set up on all donor sera, irrespective of blood group, simultaneously with other tests utilizing donor serum, *i.e.*, the serology tests and the confirmation, or reverse, ABO groupings. The capillary pipets used in measuring amounts of serum and cells are commercially obtained in order to standardize the size of the bore. Glassware is thoroughly rinsed in distilled water before it is dried so that "false hemolysis" resulting from traces of hemolytic chemical substances is avoided.

Two drops of donor serum are pipetted into each of two 10 x 75 mm. test tubes. Two drops of a 5 per cent suspension of A_1 cells are added to the serum in tube "A"; 2 drops of a 5 per cent suspension of B cells are added to the serum in tube "B". The serum-cell mixtures are shaken gently and immediately incubated in the 37° C. water bath for from 60 to 90 minutes. After incubation, the tubes are shaken to distribute the free hemoglobin evenly throughout the mixture and are centrifuged for two minutes at 1-2000 r.p.m.

The source of foreign complement for hemolysin tests performed on sera more than 24 hours old is a fresh group O serum which lacks hemolysins itself and has a positive complement control. Equal parts of the serum to be tested, the fresh group O serum, and the appropriate 5 per cent suspension of cells are placed in a tube and the screening procedure is carried out as just described.

Results of the hemolysin tests are read by macroscopic examination. Both the hemolysis evident in the supernatant serum and the size of the red cell button remaining in the bottom of the tube are noted and results are recorded as negative to 4+ as indicated below. The categories of weakly hemolytic, moderately hemolytic, and strongly hemolytic are convenient for comparing the results of the hemolysin tests with other screening procedures.

Strongly hemolytic:	4+ (complete hemolysis)
	3+ (75% hemolysis)
Moderately hemolytic:	2+ (50% hemolysis)
	1+ (25% hemolysis)
Weakly hemolytic:	tr (trace of hemolysis)
	0 (no hemolysis)

Only those donors whose sera are classified as nonhemolytic or weakly hemolytic are arbitrarily regarded as "safe" for transfusion to recipients of other blood groups. Undoubtedly many sera in the moderately hemolytic category could be safely transfused. This rigid criterion was adopted because (1) calibrated pipets are not used in measuring amounts of serum and cells, (2) it is difficult for even the same observer to agree on readings of 1+ or 2+, if such endpoints were the criterion for rejection, and (3) the number of donors acceptable by this criterion (summarized in Table 1) are sufficient for the needs of this blood bank.

TABLE I

Summary of Acceptable* Donors Based on Results of Hemolysin Screening Procedures on 1483 Groups O, A, and B Sera

Blood Group of Donors	Acceptable for Recipients of Group	Number of Acceptable Donors	Number of Sera Tested
O	A	326 (42%)	782
	B	529 (68%)	
	A, B, or AB	303 (39%)	
A	AB	407 (73%)	556
B	AB	80 (55%)	145

* Donors are acceptable if the hemolysin screening test exhibits no hemolysis or a faint tinge of hemolysis.

Test for intrinsic complement (positive complement control). Complement controls were performed on 503 donor sera to determine the advisability of such a control.

A group O serum known to contain strongly hemolytic anti-A and anti-B was inactivated for 30 minutes in the 56° C. water bath. By means of capillary pipets, equal volumes (2 drops) of the donor serum and the inactivated hemolytic serum were placed in a 10 x 75 mm. test tube. One drop of a 5 per cent saline suspension of A_1 cells and 1 drop of a 5 per cent suspension of B cells were added to the serum mixture. The tube was incubated in the 37° C. water bath for from 60 to 90 minutes. After incubation the test was shaken gently and centrifuged for two minutes at 1-2000 r.p.m. Hemolysis in this tube indicated that there was an adequate amount of intrinsic complement in the donor serum, and that the results of the hemolysin screening test were valid.

A negative control tube was also set up with each day's series of complement controls. This negative control consisted of 4 drops of the inactivated hemolytic serum and 1 drop each of a 5 per cent suspension of A_1 and B cells. The test was incubated and completed with the complement controls. There should be no hemolysis in this tube.

At the beginning of the investigation of complement controls, 2 drops of A_1 cells were added to the serum mixture instead of 1 drop each of A_1 and B cells. More than the expected number of negative complement controls were observed when group A sera were tested. An experiment was therefore carried out in which a duplicate series of complement controls were set up on group A sera. A_1 cells were added to the tubes in series "A" and B cells were added to series "B". The hemolysis in series "B" was often stronger than in series "A" and at times hemolysis was evident in tube "B" when none was seen in tube "A". The reason for this may be that some group A sera contain an amount of A group specific substance sufficient to inhibit the activity of the anti-A hemolysin

in the inactivated hemolytic O serum. From this experience the procedure was modified so that 1 drop each of A_1 and B cells were added to the complement controls.

Of the 503 sera tested, two group O, two group A, and one group B sera were shown to lack adequate intrinsic complement for hemolysis, as evidenced by negative complement controls. When these five sera were tested again for hemolysins, the second time with an added source of complement (a group O serum free of hemolysins, but with a positive complement control), none demonstrated more than a trace of hemolysis. It is realized that sera with insufficient amounts of native complement could contain potent hemolysins, but until results of a larger series prove the practical necessity for a control tube, the test is not included in our routine hemolysin screening procedure.

Results

Comparison of the hemolysin and indirect Coombs screening tests. Tests for hemolytic and "immune" agglutinins were performed on 150 group O, 133 group A, and 51 group B bloods. The results of this comparison series are summarized in Tables 2 and 3. The majority of group O sera which contained "immune" anti-A, as determined by the indirect Coombs technic, also demonstrated moderate or strong hemolytic activity, although there were five exceptions. These five sera, acceptable as "universal donors" by the hemolysin test, were titerd for "imune" anti-A antibodies as described in the section on Methods. One of the five sera contained "immune" anti-A in a titer over 1:16, the criterion for rejection by Grove-Rasmussen *et al.*[10,11] It will also be noted from Table 2 that nine group O sera would have been acceptable if screened by the indirect Coombs technic, even though they exhibited strong anti-A hemolysins. None of the 51 group B sera tested gave positive reactions in the indirect Coombs screening test, although four sera were strongly hemolytic.

TABLE 2

Relation of Anti-A Hemolytic Activity of 201 Random Groups O and B Sera to "Immune" Anti-A As Determined by the Indirect Coombs Test Following Neutralization by Group Specific Substance

Anti-A Hemolytic Activity	Blood Group	SERA TESTED BY INDIRECT COOMBS METHOD		Total
		Positive Reaction	Negative Reaction	
Nonhemolytic or weakly hemolytic	O	5	66	71
	B	0	24	24
Moderately hemolytic	O	22	27	49
	B	0	23	23
Strongly hemolytic	O	21	9	30
	B	0	4	4

There was less correlation between anti-B hemolytic activity and the presence of "immune" anti-B (Table 3) than there was between anti-A hemolysins and "immune" anti-A. Of the four weakly hemolytic group A sera which gave positive indirect Coombs test, none had "immune" anti-B titers over 1:16. However, 18 group O sera in the weakly hemolytic category were shown to contain "immune" anti-B by the indirect Coombs screening test. Five of these 18 bloods contained "immune"

TABLE 3

Relation of Anti-B Hemolytic Activity of 283 Random Groups O and A Sera to "Immune" Anti-B as Determined by the Indirect Coombs Test Following Neutralization by Group Specific Substance

Anti-B Hemolytic Activity	Blood Group	SERA TESTED BY INDIRECT COOMBS METHOD		Total
		Positive Reaction	Negative Reaction	
Nonhemolytic or weakly hemolytic	O	18	83	101
	A	4	86	90
Moderately hemolytic	O	9	10	29
	A	6	23	29
Strongly hemolytic	O	17	3	20
	A	11	3	14

anti-B in titers greater than 1:16. Even though 49 (33 per cent) of the 150 group O sera demonstrated moderate or strong anti-B hemolytic activity, and 44 (29 per cent) gave positive indirect Coombs reactions, very little *in vivo* difficulty is reported in the literature from the transfusion of group O blood to B recipients. This re-emphasizes that the dangerous component of "universal donor blood" is not fully understood.

More sera exhibited hemolytic activity than exhibited the presence of an "immune" agglutinin by the indirect Coombs procedure, *i.e.*, hemolytic activity does not invariably indicate that a serum contains "immune" antibodies. Seventy-six (51 per cent) of this series of group O sera would be rejected on the basis of anti-A and/or anti-B "immune" content; 87 (58 per cent) would be rejected on the basis of hemolytic anti-A and/or anti-B activity.

Comparison of the hemolysin and 1:200 isoagglutinin screening tests. Seventy-seven group O, 84 group A, and 38 B sera were tested for naturally-occurring isoagglutinins and for hemolysins by the technics described in the section on Methods. The results of these tests agreed generally with the larger comparison series carried out by other laboratories.[3, 9, 12] Those sera exhibiting strongly hemolytic characteristics often have saline titers over 1:200, although exceptions occur. In this series seven group O sera demonstrating strongly hemolytic and anti-A and four group O's exhibiting potent anti-B hemolysins nevertheless had saline titers less than 1:200. These strongly hemolytic sera would have been classified as low titer, safe "universal donor bloods" by the 1:200 saline dilution screening test. Two group B sera and six group A sera also demonstrated strong hemolytic activity although the saline isoagglutinin titers were less than the criterion for rejection.

Only one serum among the O, A and B bloods in the weakly hemolytic category contained saline agglutinins in a titer over 1:200. This was a group O serum with an elevated anti-A saline titer. It could be concluded that the great majority of sera classified as high-titered by the saline screening method would also be rejected by the hemolysin test.

Comparison of 2 per cent and 5 per cent suspensions of red cells in the hemolysin test. In the investigations of Chaplin *et al.*[3] 2 per cent suspensions of red cells were used in the hemolysin tests. It was suggested by these workers that greater concentrations of cells failed to demonstrate the full hemolytic activity of the sera being tested, especially if suboptimal

amounts of complement were present. Complete hemolysis was the criterion for rejection according to their technic.

The series of comparison studies described in this paper was undertaken in an effort to determine whether any sera, classified as nonhemolytic or weakly hemolytic by hemolysin tests utilizing 5 per cent cells, would demonstrate complete hemolysis if 2 per cent cells were used. Forty-eight group O, 62 group A, and 15 group B sera were chosen for study with 2 per cent cell suspensions on the basis of the nonhemolytic or weakly hemolytic results obtained when tested with 5 per cent cells. Complement controls on all sera were positive. The same A and B donor bloods were used to prepare the 2 per cent and 5 per cent suspensions of cells in saline.

Five of the 48 group O sera exhibited 2+ and 3+ hemolysis with 2 per cent A cell suspensions; none of the five was completely hemolyzed (criterion for rejection by Chaplin *et al.*). However, one of these five hemolytic sera had an "immune" anti-A titer over 1:16 (criterion for rejection by Grove-Rasmussen *et al.*). None of the group B sera revealed strong hemolysins with 2 per cent cells.

When the group O and group A sera were studied for anti-B hemolytic activity, it was shown that five group O and one group A sera exhibited a marked increase in activity when 2 per cent B cells were used. The group A serum demonstrated 2+ hemolysis and the "immune" titer was less than 1:16. One of the five hemolytic group O sera did exhibit complete hemolysis, but none of the five had "immune" anti-B titers over 1:16.

These comparison tests suggest that very few sera would be rejected by the hemolysin test using 2 per cent suspensions of cells which would be accepted as "safe" by the hemolysin technic utilizing 5 per cent cells, especially if the criterion for acceptance is no more than a trace of hemolysis when 5 per cent suspensions are used.

Discussion

The need for screening procedures is increasing in proportion to the increased number of blood donors who are receiving injections such as tetanus toxoid and T.A.B. vaccine which are known to stimulate the production of hemolytic and "immune" isoagglutinins.[4,13] The sera of women donors who have had heterospecific pregnancies may also be expected to contain "immune" varieties of anti-A and anti-B. Donors who, as patients, have received plasma or blood transfusions conditioned with group specific substances may have developed isoagglutinins with immune properties, presumably due to excesses of the specific substances left free in the plasma.[13] In addition, the "immune" agglutinins have been demonstrated in the sera of persons who have had no apparent source of sensitization.[11]

Blood reserved for the exchange transfusion of infants, as well as blood reserved for the emergency transfusion of adult recipients, should be tested for the presence of hemolytic or "immune" antibodies.[14,16,18] Those blood banks adding group specific substances to emergency transfusions as an alternative to performing screening tests are not protecting recipients from the "immune" type of agglutinin because "immune" antibodies are not easily neutralized.[7,11,17] Also, complete reliance should

not be placed on the 1:200 saline agglutinin test in selecting "safe" donors since high titers of "immune" antibodies may occur in bloods with titers of saline isoagglutinins below 1:200.[10,11,15]

The "immune" agglutinin screening test developed by Grove-Rasmussen and his associates[10,11] has certain disadvantages for wide-scale use. It is time-consuming and entails the considerable expense of group specific substances and Coombs reagent. Also, different lots of Coombs serum have been shown to vary in demonstrating the coating of red cells with anti-A and anti-B.[12] Difficulty has been experienced, at least in this laboratory, in reading the results of the indirect Coombs screening. A test which appears to be strongly agglutinated on first examination may "fade" rapidly to a weakly positive or doubtful result.

A hemolysin test would be ideal in its technical simplicity if it could be determined more surely that complement need not be added. Although Gardner and Tovey[9] concluded that the addition of foreign complement is not necessary to detect significant hemolysins, Chaplin *et al.*[3] found a higher incidence of positive hemolysin tests when complement was added, and McDermott and Muschel[12] reported that 5 per cent of the sera which they tested lacked the native complement required to demonstrate hemolysis. Of the 503 sera tested in this laboratory, 1 per cent gave negative complement controls. Differences in results may be attributed, at least in part, to variances in technic, the concentration of cells used, and the criterion for rejection. If complete hemolysis is the criterion for rejection, suboptimal amounts of intrinsic complement will represent a greater source of error than if lesser degrees of hemolysis are considered as the endpoint.

From Table 2 it is shown that out of a total of 49 group O sera demonstrating moderate hemolytic anti-A activity, 22 gave positive reactions in the indirect Coombs screening test. This supports the rejection of sera containing moderately hemolytic agglutinins. Some workers investigating screening procedures are interested in lowering the rejection rate so that large amounts of "universal" group O blood would be available to meet emergencies on the national level.[3] The ordinary hospital blood bank, on the other hand, is interested in a large supply of group-specific blood. "Universal donor blood" is reserved for occasional emergencies or for exchange transfusions, and large amounts are not needed. Thirty-nine per cent of all group O donors (Table 1) have been acceptable as "universal" donors under the conditions of the hemolysin test as performed in this laboratory. This percentage of acceptable group O donors, together with the larger percentages of acceptable groups A and B donors, has adequately met our requirements for emergency transfusions.

An attempt was made originally to incorporate the hemolysin test into our confirmation, or reverse, ABO groupings. This method was rejected for several reasons. Five per cent concentrations of red cells suppress agglutination by the naturally-occurring agglutinins. In addition, the agglutination of the cells at room temperature by the isoagglutinins seemed to inhibit the action of the hemolysins when the tests were subsequently incubated at 37° C. Adding 1 drop of the 5 per cent cells (in place of 2 drops) to the 2 drops of serum resulted in clear-cut easily read agglutination in the confirmation groupings but the addition of the

second drop of 5 per cent cells before incubation at 37° C. did not eliminate the problem of diminished hemolytic activity. McDermott and Muschel[12] suggested performing the hemolysin tests first, followed by the confirmation tests. The tubes are allowed to stand at room temperature for 30 minutes after results of the hemolysin tests are read, and the tests are then examined for agglutination and hemolysis as confirmation typings. This procedure was not adopted as our routine partly because of the disadvantages in using a 5 per cent cell suspension for reverse typings, and partly because less time is consumed in setting up two sets of tubes than is consumed in performing the tests in the same set of tubes, one after the other.

Hemolysin screenings are routinely set up on all donor sera and the results of all tests except for group AB are recorded. Groups A and B are preferred to group O for transfusion to the AB recipient.[1,18] Group A is chosen if the recipient is type A_1B; group B is the better choice for the A_2B patient to exclude the possibility of sensitization to the A_1 antigen. Another reason for the use of A or B donors for AB recipients is that O blood has two isoagglutinins while A and B blood have only one. The anti-A and anti-B saline agglutinin titers of groups B and A respectively are generally lower than those found in group O serum and are less likely to exhibit immune characteristics. Extreme caution should be used in transfusing group-specific blood to the recipient who has received heterospecific blood.[2] Serious and even fatal reactions have resulted from the destruction of the group-specific donor cells by isoagglutinins previously transfused with the heterospecific blood.[5] In most cases it is advisable to continue the transfusion of "universal donor blood" which has been screened for antibody content.

The temptation must be resisted to consider any one of the screening tests now in use as a valid standard against which to compare the results of any other screening technic. No completely satisfactory screening procedure has yet been developed. In the studies reported here, emphasis was placed on comparison of the hemolysin and indirect Coombs test for "immune" antibodies because it is felt that the titers of normally-occurring agglutinins, as would be determined by a routine 1:200 saline dilution screening test, will be sufficiently lowered by the addition of group specific substance to the donor blood.

The final test of validity for any screening test is in the uneventful transfusion of the blood classified as "safe" under the conditions of the procedure. Since the hemolysin test was incorporated into our routine processing procedures, 32 units of O and A blood have been transfused to nine patients without clinical evidence of reaction. The appropriate A or B group specific substance was added to each unit just prior to infusion. Eight pints of group O blood, found to be nonhemolytic or weakly hemolytic by the anti-A hemolysin test, were transfused to a patient whose true ABO group was never determined. This patient had suffered a hemolytic transfusion reaction from one or more of the six units received two days before admission to this hospital for treatment. Direct grouping of his cells presented a microscopic picture of approximately 50 per cent O and 50 per cent A red cells. Four infants of groups A and B were given exchange transfusions of O blood. Four AB recipi-

ents, representing surgical emergencies necessitating the transfusion of more than the anticipated amounts of blood, were given group A blood. The largest number of A units transfused to any one AB patient was nine pints. One AB recipient received two units of A blood to which A, instead of B, group specific substance was mistakenly added. No apparent reaction occurred even though the anti-B isoagglutinins were not neutralized with group specific substance before transfusion.

Summary

The procedure for a hemolysin screening test for the selection of "safe universal donors" is presented. The test is technically simple and requires no expensive reagents or equipment.

Comparison studies indicate that the great majority of sera rejected for universal use by either the 1:200 saline isoagglutinin test or the indirect Coombs screening technic would also be rejected by the hemolysin method.

Nine patients were uneventfully transfused with a total of 32 pints of heterospecific blood which had been tested and classified as "safe" by the hemolysin screening test.

REFERENCES

1. American Association of Blood Banks: Technical Methods and Procedures, revised. Minneapolis, Minn., Burgess Pub. Co., 1956.
2. Chaplin, H.: Hemolytic transfusion reaction associated with the transfusion of "dangerous universal donor" blood. Ann. Int. Med. **43:** 1334-1340, 1955.
3. Chaplin, H., Wallace, M. C., and Chang, E.: A study of isoagglutinin and hemolysin screening procedures for universal donors. Am. J. Clin. Path., **26:** 721-735, 1956.
4. Crawford, H., Cutbush, M., Falconer, H., and Mollison, P. L.: Formation of immune A iso-antibodies, with special reference to heterogenetic stimuli. Lancet, **2:** 219-223, 1952.
5. Crosby, W. H., and Akeroyd, J. H.: Some immunohematologic results of large transfusions of group O blood in recipients of other blood groups; a study of battle casualties in Korea. Blood, **9:** 103-116, 1954.
6. Dunsford, I., and Bowley, C. C.; Techniques in Blood Grouping. Springfield, Ill., Charles C. Thomas, 1955.
7. Ervin, D. M., Christian, R. M., and Young, L. E.: Dangerous universal donors. II. Further observations on *in vivo* and *in vitro* behavior of isoantibodies of immune type present in group O blood. Blood, **5,** 553-567, 1950.
8. Ervin, D. M., and Young, L. E.: Dangerous universal donors. I. Observations on destruction of recipient's A cells after transfusion of group O blood containing high titer of A antibodies of immune type not easily neutralizable by soluble A substance. Blood, **5:** 61-73, 1950.
9. Gardner, J. M., and Tovey, G. H.: Potentially dangerous group-O blood; a screening test. Lancet, **1:** 1001-1004, 1954.
10. Grove-Rasmussen, M., Shaw, R. S., and Marceau, E.: Hemolytic transfusion reaction in group-A patient receiving group-O blood. Am. J. Clin. Path., **23:** 828-832, 1953.
11. Grove-Rasmussen, M., Soutter, L., and Marceau, E.: The use of group O donors as "universal" donors. Proceedings, Fourth Annual Meeting, Am. Assoc. Blood Banks, Oct., 1951.
12. McDermott, D. A., and Muschel, L. H.: A hemolysin test for selection of universal donors. Am. J. Clin. Path., **26:** 4-12, 1956.
13. Mollison, P. L.: Blood Transfusion in Clinical Medicine. Springfield, Ill., Charles C. Thomas, 1951.
14. Prest, E., Bonnin, J. A., Simmons, R. T., and Newland, B. T. J.: Hemolytic disease of the newborn due to ABO isosentization in association with potent anti-M agglutinins. Med. J. Aust., **11:** 153-156, 1955.
15. Stevens, A. R., and Finch, C. A.: A dangerous universal donor. Am. J. Clin. Path., **24:** 612-620, 1954.
16. Vaughan, V. C.: Current status of hemolytic disease of the newborn. Postgrad. Med., **18:** 115-122, 1955.
17. Witebsky, E.: Interrelationship between the Rh system and the A B system. Blood, Spec. Issue No. 2, pp. 66-79, 1948.
18. Zoutendyk, A.: The incidence of the "dangerous" group O donor; results of the titration of one thousand consecutive sera. S. Afr. Med. J., **21:** 438-441, 1947.

EXFOLIATIVE CYTOLOGY OF THE VAGINA, THE CERVIX AND THE UTERUS*

NORMA JEAN BAKER

School of Medical Technology, Mary Hitchcock Memorial Hospital
Hanover, New Hampshire

The study of exfoliated cells dates back to the middle of the nineteenth century. Such names as Walshe, Pouchet, Luecke, Klebs, and Quincke are outstanding in the early explorations in this field. During the past decade or two, great strides have been made by workers, such as Papanicolaou, Traut, and Ayre, toward the ultimate goal—that is, the recognition of cancer in its very early stages. Despite their noteworthy progress, there is still much to be attained in the endless fight against human malignancy.

In order to better understand the exfoliative cytology of the vagina, cervix, and uterus, it is first necessary to become familiar with the anatomy of these organs.

The uterus, or womb, is a hollow, thick-walled, muscular organ oriented in the true pelvis between the bladder, in front, and the rectum, behind. In form, the uterus is more or less pear-shaped. The usual length of the adult uterus is about three inches, its greatest breadth nearly two inches, and its maximum thickness about one inch. It is divided into three parts called the fundus, the body, and the cervix.

The fundus is the free, convex end, or that portion of the uterus which lies above the points of entrance of the uterine tubes.

The body diminishes slightly in width from the fundus to the cervix.

The cervix is cylindrical and at its commencement it is sometimes marked off from the body by a slight constriction. It is about one inch in length and its lower end tapers slightly as it enters the upper portion of the vagina. The wall of the cervix is composed chiefly of dense collagenic connective tissue.

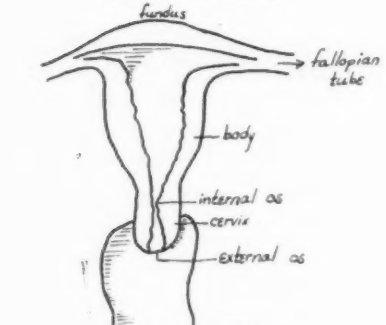
The uterine wall is composed of three layers—the serous, the muscular, and the mucous coats.

The serous coat (perimetrium) is the peritoneal covering. It covers the fundus and body and it is rather strongly adherent to the deeper underlying layers. It consists of a single layer of mesothelial cells supported by a thin connective tissue membrane.

The muscular coat (myometrium) is composed of unstriped smooth fibers and forms the major portion of the uterine wall. The muscular coat of the cervix contains more fibrous and elastic tissue than that of the body, hence its greater firmness and rigidity. It also contains many of the larger blood vessels.

The mucous coat (endometrium) in the body of the uterus is smooth and soft and covered with columnar ciliated epithelium. Simple tubular glands, also lined with ciliated epithelium, are present here. In the cervix, the mucous coat is firmer and more fibrous than in the body. It too is covered with a ciliated epithelium which passes into squamous epithelium just inside the external os (opening through which the cavity of the uterus has contact with the vagina). In addition to the un-

* Read by title—Annual Convention, Chicago, Illinois, June, 1957.



branched tubular glands, numerous slightly branched glands are found in the cervix uteri.

At each menstrual period a remarkable series of periodic changes occur which result in a shedding of the superficial parts of the uterine mucous (functional layer). A few days before menstruation begins, the mucous membrane gradually thickens and becomes more vascular, while at the same time, its surface becomes uneven. Soon the superficial parts of the mucous membrane disintegrate and hemorrhages takes place from small superficial blood vessels. Thus a hemorrhagic discharge is caused and superficial parts of the uterine mucous membrane are shed at each period. When menstruation is over the mucous membrane is rapidly regenerated.

The vagina is a musculofibrous tube about three inches in length, open at its lower end and communicating above with the body of the uterus through the cervix. At its lower end, the vagina opens into the pudendal cleft between the labia minora. The opening is partly closed in a virgin by a thin fold called the hymen.

The vaginal wall has a muscular coat composed of unstriped muscle fibers most of which are longitudinally placed. Toward the lower end, circularly disposed bundles of striped muscle fibers are found in this coat.

The mucous coat is thick and is corrugated by a number of transverse ridges known as vaginal rugae. It is covered with stratified squamous epithelium. There are also small nodules of lymphoid tissue throughout the mucous coat. No glands are present in this mucous layer.

Exfoliated cells normally found in vaginal, endocervical and endometrial aspiration smears are shed from the lining epithelium of the vagina, cervix, uterus. In vaginal smears, majority of cells encountered are of the squamous type and are derived from the epithelium of the vagina and the cervix. The cells found in vaginal, endocervical and endometrial smears may be divided into four groups.

I. Vagina-ectocervix

Squamous epithelium covering the vagina and the ectocervix consists of five zones.

a. Deepest zone, basal area, is a single layer of cuboidal or columnar cells. Cells of this zone are not normally shed and therefore are not generally found in vaginal secretions.

b. Next layer, parabasal zone, is composed of several layers of round, oval cells with a fairly large nucleus. Number of layers and size of the cells depends upon the stage of menstruation. These cells are frequently found in smears and may be divided into two groups, those poor in glycogen (as in menopause), and those rich in glycogen (as in pregnancy).

c. The third, or intermediate zone, consists of several layers of moderately flattened cells. The nuclei are relatively large, somewhat flattened and usually eccentrically placed. Exfoliated navicular cells are smaller and have a larger nucleus than superficial squamous cells. The basal, parabasal, and navicular zones have been designated as basalis in contrast to the superficialis which consists of more superficial zones.

d. The fourth zone corresponds to the stratum granulosum of the epidermis. Its cells contain distinct keratohyaline granules. This zone is not well defined and consists of flattened and densely packed cells which are strongly acidophilic and have small nuclei.

This zone has little practical cytologic significance in exfoliative cytology since its cells cannot be easiy distinguished from those of the fifth or

e. superficial zone. This layer consists of several layers of flattened cells. The nuclei are small. Some of the cells are basophilic, while those undergoing cornification are acidophilic. A certain amount of glycogen is retained in this layer.

II. Endocervix

The endocervix, including its glandular components, is covered by simple cuboidal or columnar epithelium consisting of two types of cells, the secretory or mucoid, and the ciliated. The mucoid cells are more numerous and their nuclei are basally located. The ciliated cells are scattered singly or in small groups between the mucoid cells and are more superficially located. The height and form of the secretory cells depend upon their function which in turn is related to such factors as age, stage of menstrual cycle, etc. Both types of cells are found in endocervical smears. Exfoliated mucoid cells may retain their cuboidal or columnar form or may become more rounded. As mentioned, the nucleus is often basally located and is usually round or oval and the cytoplasm shows irregular vacuolation. When in clusters, the cells are usually rounded and have distinct cellular borders.

The ciliated cells generally retain their cuboidal or low columnar form and they may be identified by their cilia and dense cuticular membrane. A small perinuclear vacuole is often present. Ciliated cells appear singly or in small clusters and sometimes in palisade arrangement. Multinucleation is not uncommon. A peculiarity seen chiefly in ciliated cells is a knob-like protrusion at the distal pole of the nucleus. This has been interpreted as a dense concentration of chromatin, staining deeply with hematoxylin.

III. Endometrium

The epithelium lining of the endometrium, including glands, consists of secretory and ciliated type cells. The secretory cells are of the mucoid variety and they have a cuboidal or low columnar form with a basally located nucleus.

The ciliated cells are usually inconspicuous and can be better demonstrated with special techniques. Beneath the surface epithelium and between the glands is the uterine stroma consisting of relatively small cells. These cells sometimes resemble the embryonic mesenchymal type.

The nuclei of exfoliated endometrial cells are round or oval and have a fine chromatin network. The identification of endometrial cells is often difficult as they may be confused with endocervical cells or histiocytes. The endometrial cells tend to be smaller than the endocervical cells and they tend to form denser, more compact groups in which the cell outline is not always clear. Clusters of endometrial cells often exhibit characteristic patterns which facilitate identification. The existence of cyclic modification of endometrial cells is apparent.

IV. Fallopian tubes

The epithelial lining of the uterine tubes consists of the same two types found in the remainder of the female genital tract—the ciliated and secretory. The ciliated cells are more numerous and are much better developed than in the endometrium and the endocervix. The normal exfoliative cytology of the uterine tubes and the extent of exfoliation are practically unknown.

There is an area of atypical cytology of the vagina, endocervix, and the endometrium which is non-malignant and therefore will not be covered. However, atypical cells and structures may be confused with malignant cells, so extreme care must be taken to avoid confusion and faulty diagnosis.

Cancer is a fascinating problem, both from biological and medical points of view. It focuses attention on cells both normal and malignant, for neither can be appreciated without knowledge of the other.

A clear cut definition is difficult to give. The usual definition is: cancer is a malignant neoplasm.

Cancer is a Latin word meaning a crab. The extensions into the surrounding skin from a primary cancer were thought to bear some resemblance to the legs extending out from the body of a crab. The word malignant as used here, implies resistance to treatment, a strong tendency to grow worse to fatal termination.

Neoplasm signifies a new growth. Cancer is truly a new growth insofar that the cells, having become cancerous, grow in a new and different way from their nonmalignant ancestors. Neoplasm does not refer solely to a tumor, but to any swelling. Some neoplasms are malignant, some benign. Carcinoma is an epithelial cancer. Sarcoma refers to malignant growths found in the "between cells" (mesothelial cells) of bone and of connective tissue, making up most of the flesh of the body. Mixed tumors or malignant growths are derived from cells of more than one germ layer, i.e., ectoderm and mesoderm. Teratoma is a malignant neoplasm which includes multiplying cells from all germ layers (ectoderm, mesoderm, and endoderm).

It is convenient to use the term cancer in a general way as meaning any one of the many malignant neoplasms.

Benign growths or tumors resemble the tissue of origin much more closely than do malignant ones. Their neoplastic cells have a more uniform appearance and the pattern of arrangement may at once call to mind the parent tissue.

What is the difference between a benign growth and a malignant one? It has been suggested that some chemical substance or physiological attribute may find expression in malignancy and may be absent in the benign growth.

Cancers consist of malignant cells which are served by nonmalignant stroma made up of connective tissue, blood vessels, nerves, and lymphatics.

Cancer cells are abnormal in comparison with the normal cells from which they are derived. These cells fail in performance of the duties ably performed by their normal parent cells. For example, malignant fibroblasts may form a few fibers, malignant bone cells, a little bone and so on. They increase in number in an unruly fashion partly or wholly released from the usual restraints. They live only as useless, highly dangerous parasites in a cellular community. Cancer cells have the ability to invade nearby tissues and spread by metastasis to other distant locations in which they give rise to secondary cancers.

Cancer cells seem to be privileged above all others. They eat other cells out of house and home. They are a privileged yet antisocial race of cells because they act as a trap for the available amino acids.

The criteria of malignancy in exfoliative cytology are based on changes in structure of individual cells as well as their altered interrelationships. Some of the nuclear changes are disproportionate enlargement of the nucleus increase in chromatin content, structural abnormalities, as elongation, indentation, lobulation, budding; enlarged or increased nucleoli; and multinucleation. Abnormal mitotic figures, marked thickening of the nuclear membrane, and also vacuolation and fading of the nucleus; all these are changes which bring cells exhibiting such under suspicion of malignancy.

Cytoplasmic changes must also be examined. Changes in the staining reaction (certain cells may exhibit a marked basophilia or acidophilia), cytoplasmic inclusions as pigment granules, leukocytes, marked vacuolation are suspicious features found.

Changes in the cell as a whole must be noted: enlargement beyond normal range, abnormal shapes, cellular degeneration, and necrosis.

Criteria of malignancy is also based on the inter-relationships of the cells as a whole. Irregularity of pattern, marked variation of size of nuclei and cells of the same type within a cluster, lack of distinct cell boundaries, dense grouping and crowding of cells and nuclei, engulfment of one cell by another, grouping of cells into characteristic patterns (as rosette formation) and pronounced stratification of cells are criteria of malignancy.

There are several indirect criteria which are important in malignant growth, the presence of old fibrinated blood, large aggregations of lymphocytes, and polymorphonuclear leukocytes.

Neoplastic disease in the female genital system ranges from obvious carcinoma of the cervix to the obscure ovarian tumor. One of the three commonest causes of death from cancer in women is from cancer of the uterus. The epithelial tissues of this organ, with their susceptibility to endocrine influences, and surges of growth repression during and after pregnancy are particularly liable to the development of cancer.

To understand carcinoma of the uterus properly it is essential to realize that we are dealing with two main types of cancer—those which originate from the cervix and are epidermoid in character, and those of the corpus, the endometrial adenocarcinomas.

From the cytological standpoint the epidermoid carcinomas of the cervix may be divided into two distinct groups, one of which includes the early and the other the more advanced stages of malignancy.

In the early stages the morphological changes of the exfoliated malignant cells are limited almost entirely to the nucleus. The most characteristic of these are disproportionate enlargement, irregularity in form and outline, hyperchromasia, and multinucleation. The cytoplasm may exhibit some structural modifications, such as vacuolation, either perinuclear or generalized, and acidophilia.

In the more advanced stages of squamous cell carcinoma of the cervix the cytological changes are more pronounced and reflected not only in modification of the nuclear structure but also in that of the cytoplasm and the cell as a whole. An elongation of the cells is one of the most characteristic features. Cells may become spindle-shaped, resembling smooth muscle fibers. Others may acquire a tadpole appearance or other bizarre forms. Many of these cells are acidophilic and show an affinity for eosin or Orange G. The diagnosis of malignancy should be based primarily on *coexisting* nuclear abnormalities as well as the abnormal cell as a whole. The grouping of cells into large clusters (the individual cells of which there is variation of size) and stratification of the cells may also be noted. Mitosis is occasionally observed.

In adenocarcinoma of the endometrium, the exfoliated malignant cells are usually smaller and more densely grouped than those of the squamous type. The cytoplasm may show vacuolation, which is often extreme. The vacuoles are not infrequently filled with leukocytes in various stages of degeneration. The nuclei are ovoid or round or may show irregularity in form. In some cases, the nucleoli are greatly enlarged, and very prominent. Hyperchromasia is not marked. The nuclei may assume an eccentric position, particularly in cells having large vacuoles. Exfoliated adenocarcinoma cells tend to become rounded, losing their original columnar or cuboidal shape. There is some elongation. Differences in size of cells and nuclei, though often marked, are generally less pronounced in adenocarcinoma than in the epidermoid type. Degeneration and necrosis of adenocarcinoma cells is common. A pure endometrial aspiration is desirable for the detection of early uterine cancer.

There are several methods of collecting these specimens. In obtaining a vaginal aspiration, a slightly curved glass pipette, about 6 inches in length with a rounded tip and a small opening and equipped with a strong rubber bulb for producing suction is used. The patient is placed

upon an examining table in the lithotomy position. The labia are separated and the bulb compressed. The glass pipette is introduced into the posterior fornix of the vagina. The pressure on the bulb is then released and the suction produced serves to aspirate vaginal fluid with its cellular content into the glass tube. While aspiration is in progress, the tip of the tube is moved from one side to the other so that all parts are sampled. The pipette is then withdrawn, and the vaginal material is expressed and spread upon the surface of a clean slide. The material is spread over the slide and then the slide is immediately plunged into a solution of equal parts of 95% alcohol and ether. Drying of the smears should be carefully avoided as it results in the loss of the sharp outlines of the cells and in a change in the staining reaction. The smears may be kept in this fixative for a long period of time.

In the "surface-biopsy" cervical scraping, a wooden spatula or a cotton swab is used. The end of the spatula is placed in the cervical os, and a gently rotation permits a light scraping of the entire squamo-columnar junction.

With the patient in the lithotomy position, the vaginal and cervix specimens are obtained as described. The vagina, cervix and cervical canal are then wiped clean and painted with a disinfectant solution. The anterior lip of the cervix is grasped with a tenaculum. An antrum or a laryngeal cannula attached to a sterile, closed ten cc. syringe is then inserted through the endocervix into the endometrial cavity, about one half inch from the fundus. Suction is created by withdrawing the plunger almost to the end. At this point, the syringe is detached, closed, reattached and suction reapplied. The cannula is withdrawn a little with each repetition of suction. After the third suction, the cannula must be withdrawn from the uterus, and the contents of the cannula are forcibly expelled on the end of a glass slide by blowing air through the cannula.

Staining of smears for cytologic diagnosis of cancer is directed toward three chief objectives.

1. Definition of nuclear detail—because of widespread nuclear abnormalities of cancer cells and their diagnostic significance, this is important.

2. Transparency—important because of varying thicknesses and frequent overlapping of cells.

3. Differentiation of cells—as between acidophilic and basophilic.

Papanicolaou staining is one of the more popular techniques. After fixation, transfer the slides without drying, directly from alcohol-ether to 80% alcohol and run down through 70% and 50% alcohols to distilled water. Stain in Harris hematoxylin and rinse in distilled water, gently to prevent washing off smears. Dip in .25% aqueous solution of hydrochloric acid and then place in running tap water. Run through 50%, 70%, 80%, and 95% alcohols. Stain in Orange G and rinse in 95% alcohol (two changes). Stain with EA 36. This contains light green-yellowish, Bismark brown, and eosin yellowish. Now rinse in 95% alcohol (three changes) and dehydrate and clear by running through absolute alcohol and xylol, equal parts, and xylol. Any neutral medium is satisfactory for mounting. The results of this stain—nuclei—blue purple and hematoxylin, red blood cells—orange, cornified cells—red, pink, orange, basophilic cells—blue, green.

The task of recognizing cancer cells when present in small numbers, as in early cases may be facilitated by omission of counterstaining. Staining the nuclei with hematoxylin stain is sufficient. Cytoplasm of cells and blood stain very faintly and variations in size and structure can be readily detected. The same smear can be subsequently counterstained with cytoplasmic stains in order to make a complete study of all cellular elements.

The Ayre method follows the Papanicolaou method down through the hematoxylin staining and subsequent treatment with hydrochloric acid and carbonate until blue. Rinse in cold running tap water and then in 50%, 70%, 80%, 95% alcohols. Stain in EA 36 and complete as in Papanicolaou.

Another method is the hematoxylin eosin-indigo carmine. Rinse the slides in 95% alcohol and transfer to distilled water. Stain with Harris hematoxylin and wash in running tap water. Immerse in a mixture of absolute alcohol, water, strong ammonia. Then stain overnight in an indigo carmine, eosin yellowish, water, and chloroform mixture. Rinse in tap water and dehydrate with 70%, 90% and absolute alcohol. Clear in xylol and mount. Results—nuclei of epithelial cells stained gray, cytoplasm violet. Cornified cells are brilliant pink, and erythrocytes pink.

In Shorr's stain, the slides are carried from the fixing solution through the alcohols to water and then stained in Harris hematoxylin. After washing in water, stain in a solution of Biebrich Scarlet-Orange G, water, and glacial acetic acid, phosphotungstic acid, and water. Now wash in water and stain in a solution of fast green and acetic acid and then without washing, differentiate in acetic acid. Dehydrate in the usual manner, clear in xylol and mount in dammar xylol. Results . . . cornified cells—orange, red, noncornified cells—green.

In this paper, only an introduction into this vast field of research has been made, commencing with the anatomy of the female genital tract and its normal cytology, followed by a general discussion of cancer and the criteria of malignancy, and then by a specific listing of the adbormalities found in malignant exfoliated cells of the uterus, cervix, and vagina. Finally, the various methods of collecting and staining the aspirated material were reviewed.

BIBLIOGRAPHY

1. Ayre, Ernest. *An Atlas of Cervical Cytology*, reprinted from "What's New" (July, August, September, October). Chicago: Abbott Laboratories, 1948.
2. Brash, J. C. and Jamieson, E. B. *Cunningham's Text Book of Anatomy*, 7th ed. New York: Oxford University Press, 1937.
3. Cowdry, E. V. *Cancer Cells*. Philadelphia: W. B. Saunders Company, 1955.
4. Greisheimer, Esther M. *Physiology and Anatomy*, 6th ed. Philadelphia: J. B. Lippincott Company, 1950.
5. Gurr, Edward. *A Practical Manual of Medical and Biological Staining Techniques*. London: Leonard Hill Limited, 1953.
6. Ham, Arthur Worth. *Histology*, 2nd ed. Philadelphia: J. B. Lippincott, 1953.
7. Nettleship, Anderson. *Basic Principles of Cancer Practice*. Baltimore: The Williams-Wilkins Company, 1952.
8. Papanicolaou, George N. *Atlas of Exfoliative Cytology*. Cambridge, Mass.: Harvard University Press, 1954.
9. Papanicolaou, George N. and Traut, Herbert F. *Diagnosis of Uterine Cancer by the Vaginal Smear*. New York: E. L. Hildreth and Company, 1943.

THE ROLE OF A MEDICAL TECHNOLOGIST IN A HEART STATION AND ITS LABORATORY*

GEORGE C. GUNDLACH, JR., M.A., MT (ASCP)
Vanderbilt University Hospital, Nashville, Tennessee

In most hospital situations the heart station and its laboratory is separate from the other service laboratories, each performing a service to the patient and staff, and each having a separate group of technologists. Many hospitals do not have a heart station where electrocardiographic studies are made so that this important tool in the diagnosis of cardiac disease is not readily available to the patient.

It is the purpose of this paper to show how other laboratories can function as an adjunct to a heart station, and to outline some of the procedures which are used in the Vanderbilt University Hospital Heart Station and its Laboratory. Further, it is the purpose of this paper to show that the medical technologist should have as a part of his training a course in electrocardiographic instrumentation so that he will be familiar with the instruments and their functions in the heart station-laboratory situation.

At Vanderbilt University Hospital, the Heart Station is a part of the Department of Clinical Physiology which functions to give the patient and staff the most efficient and modern techniques available in the diagnosis of cardiac disease and diseases of the cardiovascular and pulmonary systems. It routinely does twenty to thirty electrocardiograms per day as well as spirograms, electrolyte balance studies on patients in cardiac and renal failure, oxygen saturation studies, dye dilution curve studies and pressure tracings on patients who come in for cardiac catheterization. There are also many research projects in progress which deal with the heart and its diseases.

The routine laboratory procedures performed by this laboratory are numerous and provide the technologist with a wide range of experience not only in techniques but also in instrumentation. To outline all of the procedures of this laboratory would require a large volume and more time than the author has to devote to the writing of this paper, so he has chosen the most important ones from the standpoint of the physician, the technologist, and the patient.

The group of procedures used for electrolyte balance studies on patients in renal and cardiac failure consists of the daily analysis of urine, feces, vomitus, and any therapeutic losses of fluid from the body for volume, chlorides, sodium, and potassium. In addition to these, aliquots are tested for NPN, phosphorus, creatinine, and creatine.

Chlorides are determined by the method of P. H. Sanderson which is an amperometric argentometric titration in which the removal of protein is unnecessary.[1]

Five milliliters of 50% glacial acetic acid are placed in a 10 ml. beaker. To this is added the measured sample (usually 1 ml.) and a drop or two of capryl alcohol. This is titrated with a 0.1 normal $AgNO_3$ solution from a Conway burette which is attached to a direct reading potentiometer.

* Read before the Annual Convention, Chicago, Illinois, June 1957.

The advantage of this type of instrument over other potentiometric methods is the elimination of the troublesome salt-agar bridges without sacrificing either the speed or accuracy of the determination.

Sodium and potassium determinations are made with the flame photometer. Flame photometric techniques need no introduction to the technologist; however, it is important to mention that the accuracy of these determinations must be checked constantly as minor changes in the excreted sodium, potassium, or chlorides will affect the management of the patient by the physician.

In all electrolyte balance studies the results are given in meq/liter and in total meq excreted. Graphic presentation of the results also aids the physician in the management of this type of patient as it shows at a glance the degree of positive or negative balance of the electrolytes for any given day of treatment.

The procedures used in cardiac catheterization studies consist of oxygen saturation analyses of whole blood samples, dye dilution curves for cardiac output studies and cardiac vascular pathway studies, and pressure tracings to determine the blood flow patterns. Each of these procedures plays an important part in the total success of a cardiac catheterization study and no one of them alone can be used to accurately diagnose the cardiac anomally. The cardiac catheterization in itself is a minor surgical procedure and is carried out by a skilled physician, but he is dependent upon the assistance he receives from the qualified technologists and/or doctors who make up the team to secure the necessary blood samples, pressure data, and dye dilution curves for the successful accurate analysis of the results of the procedure. In the catheter room, aside from the operator, there is a minimum of three persons, each having a special function. One acts as the recorder and writes the protocol of the procedure as well as receiving the blood samples from the operator and/or the person operating the Sanborn Polyviso. It is his responsibility to see that the samples are properly labeled as to site, time, and number, as well as sealed with a mercury cap and passed into the laboratory in the adjacent room. The person operating the Sanborn Polyviso, in addition to securing the arterial blood samples, secures the tracings of the cardiac and arterial pressures and the dye dilution curves. The third person in the room acts as a circulating assistant and monitors the visual electrocardiogram to inform the operator of changes in the type of heart activity (premature beats).

Once the catheter and arterial blood samples are secured and passed into the laboratory, they are put into a beaker of ice and the laboratory portion of the catheterization team takes over.

The laboratory portion of the cardiac catheterization team consists of at least two and preferably three technologists. The first is the recorder of the samples and results. The second (sometimes the first also does this) prepares the samples for reading in the Beckman Spectrophotometer Model B, and cleans the cuvettes after the results have been recorded. The third person reads the Spectrophotometer. When only two persons comprise the laboratory team, the first manipulates the samples and the records while the other reads the Beckman.

The technique used for oxygen saturation studies on whole blood

using spectrophotometric methods is the one devised by Nahas which uses the spectral relationship of hemoglobin to oxyhemoglobin to determine the per cent saturation.[2] The procedure is to hemolyze the sample with 1/100 of its volume of a saturated saponin solution and to introduce a part of it into three chambers of the Nahas cuvette. The fourth chamber is used as a blank and is either filled with distilled water or left empty (it is preferable to fill the chamber with water). The cuvette is then placed into the sample chamber of the Beckman with the blank in the reading position. The positioning of the cuvette must always be the same for each successive reading as the optical density of the lucite parts of the cuvette may be different and readings of the reversed cuvettes would be inaccurate. After the dark current has been balanced out with the wave length set at 505Mu the scale is set at 0 optical density with the slit adjustment. Each chamber is read at this wave length and then is repeated at a wave length of 605Mu. Duplicate readings are made of these at each wave length. These should check within five-thousandths of an optical density unit. An initial blood sample is used to make the standard curve for the remaning samples and is treated as follows:

1. It is divided into two parts of about two and a half ml. each.
2. One part is reduced by mixing it with sodium hydrosulfite (15-20 mg./ml. of sample, this is measured by using a long 18 gauge needle, 2 dips from the bottle per ml. of sample).
3. The other part is oxygenated by introducing 100% oxygen into the syringe and equilibrating (3 times for five minutes each with at least two volumes of oxygen).
4. Both samples are hemolyzed with 1/100 volume saponin solution.
5. Both parts are then read as previously described. From the ratios of the 605/505 readings the standard curve is made. A new curve must be made for each patient.

The standard curve consists of the line between the ratio of 605/505 readings of the reduced part of the sample plotted as 0% saturation and the 605/505 readings of the oxygenated part of the sample plotted as 100% saturation as plotted on linear graph paper. In the ideal condition each curve should be superimposed upon the others. However, if the curves are parallel and are close together the results are valid and the amount of error is negligible. If the curves cross, the standard curve should be repeated. The per cent saturation of the numbered samples is read directly from the standard curve. This method is employed because it combines the accuracy of the Van Slyke manometric gas analysis apparatus with speed without entailing any appreciable increase in the manipulation of the sample. Twenty samples can be run in triplicate within three hours by this method, whereas the same number of determinations (60) would take three eight-hour days on the Van Slyke.

The purpose of the dye curve studies is to quantitatively describe the output of the heart and cardiac circulatory pathway of the blood. This is done by injection and dilution techniques using a photoelectric densitometer in conjunction with the Sanborn Polyviso, as developed by Milnor et al,[3] and modified by Newman et al. The procedure consists of injecting a given amount of dye (Indigo Carmine)[4]—1 to 2 cc., through the catheter into the venous blood system while a sampling,

constant speed syringe pump is withdrawing a sample from a peripheral artery through a polyethylene cuvette to which is attached the photoelectric cell of the densitometer. The densitometer sensitivity of changes in light transmission is linearly transmitted to the galvanometer of the Sanborn and a direct writing record of the curve is made (Figure 1). This technique is used because of the ease with which several repetitions of the curve may be made. There is only the need for the dye to be thoroughly mixed throughout the blood supply in order to secure a new base line prior to the next curve. Once the curves are made a calibration is made by thoroughly mixing a sample of blood with the dye and passing it through the cuvette, measuring the galvanometer deflection and the concentration of dye in the plasma spectrophotometrically. It is necessary to establish at least three points of the curve to accurately determine the calibration.

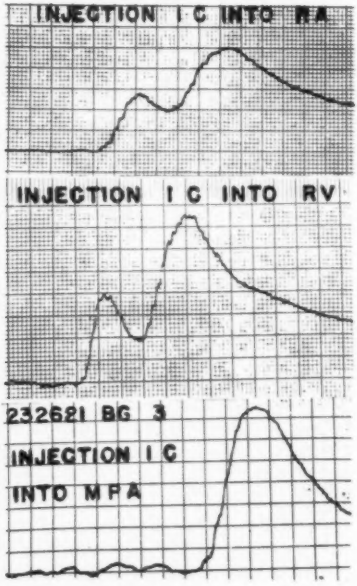


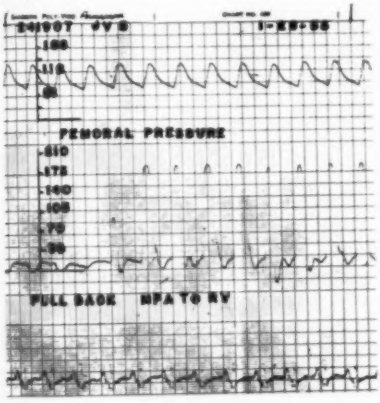
Figure 1—Serial dye dilution curves on a patient showing a right-to-left shunt at the ventricular level. Injection into main pulmonary artery gives a normal curve.

The pattern of the curve determines the cardiac pathway in that a normal curve is smooth and even with no irregularities other than respiratory variations which are easily recognizable. When there are secondary peaks on the curve, the indications are that there are variations in the cardiac blood pathway. If the secondary peak is on the ascending limb of the curve, the blood path is shunted from the venous side of the heart to the arterial side without passing through the lungs. However, when the secondary peak is on the descending limb of the curb the path is shunted from the arterial side of the heart to the venous side.

The original curve is then replotted on semi-log paper and the area under the curve is determined. By applying the calibration data to this area the cardiac output is determined.

The pressure tracings are made on the Sanborn Polyviso by applying strain gauges to the catheter and/or the arterial puncture needle hubs and recording. These are taken simultaneously in order to make direct comparison of the arterial and cardiac pressures. The position of the catheter is recorded on the tracing. The catheter may be moved during the recording from a position further advanced to one less advanced in order to take differential pressure changes within the heart. These are called "pullback" tracings (Figure 2). After the procedure is completed, the strain gauges are calibrated by measuring the pressure in millimeters of mercury required to deflect the galvanometer stylus the same as the calibration input current did. By applying the calibration to the pressure tracings, the maximum, minimum, and the mean pressures may be obtained for each tracing.

The complete information from each of the foregoing procedures is then compiled into a graphic presentation on a cardiac diagram (Figure 3). From this a diagnosis can be made by the patient's doctor in consultation with the surgeon and the laboratory director. It generally takes about six hours to complete the analyses and computations necessary for a cardiac catheterization study.



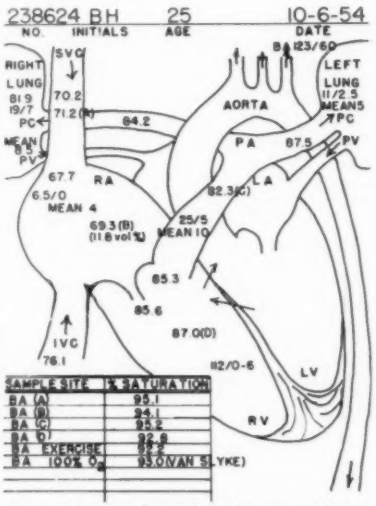


Figure 2—Pullback pressure tracing. Catheter tip is moved from the main pulmonary artery to the right ventricle. Simultaneous femoral arterial pressures and monitor electrocardiogram are also recorded. Sudden increase in pressure as the catheter tip enters the right ventricle indicates pulmonary stenosis.

Figure 3—Graphic presentation of cardiac catheterization data. Lettered saturations within cardiac diagram are simultaneous samples with corresponding lettered saturations from arterial puncture (lower left). Intra-cardiac pressures were taken simultaneously with arterial pressures. Results of analysis indicates a ventricular septal defect with a bilateral shunt.

It is evident from the foregoing procedures which are used in the Vanderbilt University Hospital Heart Station and its Laboratory, that the technologists working in the department must have training in instrumentation dealing with galvanometric measurement of pressures, dye concentration, and other special techniques used in the cardiac diagnostic laboratory as well as a thorough understanding of the more routine clinical laboratory procedures.

Because the author was employed without this training in galvanometric instrumentation, he found it necessary to spend much of the first six months of employment learning how each of the instruments worked and why they gave valid results. Had he had this training in medical technology school, much of this time could have been more productive and used to a better advantage. Therefore, it is the opinion of the author that a course in the use of galvanometric procedures such as those used in electrocardiographic instruments, and a course in instrumentation for these procedures be introduced into the curriculum of the medical technologist schools. The advantages of such a course to the technologist would be great because it would open to him many opportunities for study and improvement in the techniques he now uses by the introduction of electronic measurement. To the hospital employing a technologist with such training, the advantage would come with the opening or increasing the services rendered to the patient needing electrocardiographic studies and cardiac diagnostic laboratory tests.

With the increasing number of patients having cardiac disease, there is an increasing need for personnel trained in cardiac laboratory instrumentation and procedures. Perhaps the best place to secure such training, as far as the technologist is concerned, is the medical technologist school. However, few schools are at present equipped to give adequate training in this type of instrumentation and procedure so that the technologist must resort to "on the job" training which is not only more difficult but also more costly to the institution providing it.

It has been shown that certain procedures of a heart station require the services of a laboratory and that these two units can and should work together as a team. It has further been shown that these procedures require a technologist with special training in the use of galvanometric instruments and the interpretation of the results obtained. It has been suggested that this special training be incorporated into the curriculum of the medical technologist school in order to provide more trained personnel for the cardiac diagnostic laboratory.

REFERENCES

1. Sanderson, P. H.: Potentiometric determination of chloride in Biological fluids. Biochemical Journal, **52:** 502, 1952.
2. Nahas, Gabriel G.: Spectrophotometric determination of hemoglobin and osyhemoglobin in whole hemolyzed blood. Science, **113:** 723, 1951.
3. Milnor, William R., et al: A photoelectric ear densitometer for continuously recording the arterial concentration of T-1824 in the dye dilution method. Circulation Research, **1:** 117, 1953.
4. Lacy, William W., et al: The use of indigo carmine for dye dilution curves. Circulation Research, **3:** 570, 1955.

It is evident from the foregoing procedures which are used in the Vanderbilt University Hospital Heart Station and its Laboratory, that the technologists working in the department must have training in instrumentation dealing with galvanometric measurement of pressures, dye concentration, and other special techniques used in the cardiac diagnostic laboratory as well as a thorough understanding of the more routine clinical laboratory procedures.

Because the author was employed without this training in galvanometric instrumentation, he found it necessary to spend much of the first six months of employment learning how each of the instruments worked and why they gave valid results. Had he had this training in medical technology school, much of this time could have been more productive and used to a better advantage. Therefore, it is the opinion of the author that a course in the use of galvanometric procedures such as those used in electrocardiographic instruments, and a course in instrumentation for these procedures be introduced into the curriculum of the medical technologist schools. The advantages of such a course to the technologist would be great because it would open to him many opportunities for study and improvement in the techniques he now uses by the introduction of electronic measurement. To the hospital employing a technologist with such training, the advantage would come with the opening or increasing the services rendered to the patient needing electrocardiographic studies and cardiac diagnostic laboratory tests.

With the increasing number of patients having cardiac disease, there is an increasing need for personnel trained in cardiac laboratory instrumentation and procedures. Perhaps the best place to secure such training, as far as the technologist is concerned, is the medical technologist school. However, few schools are at present equipped to give adequate training in this type of instrumentation and procedure so that the technologist must resort to "on the job" training which is not only more difficult but also more costly to the institution providing it.

It has been shown that certain procedures of a heart station require the services of a laboratory and that these two units can and should work together as a team. It has further been shown that these procedures require a technologist with special training in the use of galvanometric instruments and the interpretation of the results obtained. It has been suggested that this special training be incorporated into the curriculum of the medical technologist school in order to provide more trained personnel for the cardiac diagnostic laboratory.

REFERENCES

1. Sanderson, P. H.: Potentiometric determination of chloride in Biological fluids. Biochemical Journal, **52:** 502, 1952.
2. Nahas, Gabriel G.: Spectrophotometric determination of hemoglobin and osyhemoglobin in whole hemolyzed blood. Science, **113:** 723, 1951.
3. Milnor, William R., et al: A photoelectric ear densitometer for continuously recording the arterial concentration of T-1824 in the dye dilution method. Circulation Research, **1:** 117, 1953.
4. Lacy, William W., et al: The use of indigo carmine for dye dilution curves. Circulation Research, **3:** 570, 1955.

AMENDMENTS TO BY-LAWS
AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Adopted June 24, 1957

BY LAWS

ARTICLE IV
Membership

Section 1. The membership of this Society shall consist of the following classes:

(a) Active Member. Any person who (1) is registered M.T. (ASCP) by the Registry of Medical Technologists of the American Society of Clinical Pathologists and is in good standing with that Registry, or (2) possesses a master's or higher degree in microbiology, biochemistry, hematology, histology, parasitology, or serology from an accredited college, or (3) holds a certificate in chemistry or microbiology from the Registry of Medical Technologists of the American Society of Clinical Pathologists, and is in good standing with that Registry, or (4) holds a limited certificate in histologic technique from the above mentioned Registry and has ninety or more semester credit hours from an accredited college of which at least twenty-seven hours are in science including chemistry and/or biology, with lecture and laboratory in each, shall be eligible for active membership in this Society provided, further, that he is an active member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An active member shall have all the rights and privileges of this Society.

(b) Affiliate Member. Any person who holds a certificate from and is in good standing with the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists without meeting the requirements for active membership in this Society shall be eligible for affiliate membership in this Society provided that he is a member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An affiliate member is entitled to all the privileges of membership in this Society with the exception of voting, holding office, and serving on any Board or standing or special committee of this Society.

(c) Student Member. Any person enrolled in an approved School of Medical Technology or in an under graduate program which is pre-requisite to enrollment in an approved school is eligible for Student Membership in this Society. He may enjoy this membership until he becomes a registered M. T. (ASCP) by the Board of Registry of Medical Technologists of ASCP or until he becomes ineligible to take the examination for such registration. Upon successful completion of the Registry examination he shall automatically be billed for Active Membership in this Society. A student member is entitled to all the privileges of membership in this Society except voting, holding office, and serving on any Board or standing or special committee of this Society.

(d) Honorary Member. Any person who has made an outstanding contribution to the field of laboratory medicine by his research or by his service to the profession of medical technology may be recommended for honorary membership in this Society. Recommendations for honorary membership may be made by a constituent society or by the Board of Directors. A recommendation accompanied by qualifications shall be sent to the recording secretary of this Society not less than thirty days before the Annual Session. Honorary members shall be elected by a two-thirds vote of the House of Delegates. They shall be elected every third year beginning in 1954. The year when honorary members are elected each constituent society and also the Board of Directors shall be entitled to present the name of only one candidate for this honor. Honorary members shall not exceed in number one per cent of the active members of the Society in good standing at the time of their election to honorary membership. An honorary member shall be informed of his election by the recording secretary who shall send him a certificate of honorary membership signed by the president and the recording secretary. He shall enjoy all the privileges of membership except voting, holding office and serving on any Board or standing or special committee of

this Society. He shall be exempt from payment of annual dues.

(e) Corresponding Member. Any person who is resident of, and employed as a medical technologist in, a foreign country, who is in good ethical standing, and who is a member of a government—and/or medical association—recognized association of medical technologists in that country, shall be eligible for Corresponding Membership in this Society. He shall make application through the association of which he is a member. A corresponding member shall enjoy all the privileges of this Society except voting, holding office, serving on any Board or standing or special committee of this Society or any of its activities from which he might be excluded by laws governing aliens. A corresponding member shall forfeit all privileges of membership upon changing his place of residence or employment to a geographical area where there is a constituent society of this Society.

Section 2. Application for active or affiliate membership in this Society shall be made on a form prescribed by this Society. The transmittal to the executive secretary of a list of active and affiliate members in good standing in a constituent society, together with their annual dues shall establish them as members of this Society.

Section 3. Application for student membership in this Society shall be made on a form prescribed by this Society. The transmittal of this application to the executive secretary of this Society together with the required amount of dues shall establish him as a student member of this Society.

Section 4. Any member of this Society whose conduct is detrimental to this Society may be expelled by a two-thirds vote of the House of Delegates provided he has been given thirty days notice, signed by a majority of the members of the Board of Directors and sent by registered mail, stating the charges filed against him and informing him of the date of the meeting when his expulsion will be voted upon. The member so charged shall have the privilege of appearing before the House of Delegates and he may present his defense in person or through other active members of the Society of his selection.

Section 5. Any person who has been expelled from membership may be reinstated upon adoption by a two-thirds vote of the House of Delegates of his application for membership, provided the same is accompanied by annual dues to this Society plus a reinstatement fee of five dollars ($5.00).

ARTICLE V

Code of Ethics

Being fully cognizant of my responsibilities in the practice of Medical Technology, I affirm my willingness to discharge my duties with accuracy, thoughtfulness, and care.

Realizing that the knowledge obtained concerning patients in the course of my work must be treated as confidential I hold inviolate the confidence (trust) placed in me by patient and physicians.

Recognizing that my integrity and that of my profession must be pledged to the absolute reliability of my work, I will conduct myself at all times in a manner appropriate to the dignity of my profession.

ARTICLE VI

Meetings

Section 1. The Society shall hold an Annual Session which shall include one or more general or scientific meetings of the members and not less than two meetings of the House of Delegates at least two of which shall be held on different days. The Annual Session shall be held at the time and place determined by the House of Delegates at any preceding Annual Session. Emergency changes concerning the time and place of an Annual Session may be made by the Board of Directors.

Section 2. The Board of Directors shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary business and shall hold a meeting within the two weeks prior to the first meeting of the House of Delegates at each Annual Session.

Section 3. The Advisory Council shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary

business and shall hold a meeting within two weeks prior to the first meeting of the House of Delegates of each Annual Session.

ARTICLE VII
Finances

Section 1. The annual dues for active and affiliate membership in this Society shall be eight dollars ($8.00) per annum.

Section 2. (a) Dues for student membership in this Society shall be $3.00 per annum. (b) Any dues paid by a student member who becomes eligible and applies for active membership prior to January 1 will be refunded on the basis of $.25 per month for any overlapping period retroactive to July 1.

Section 3. The dues for corresponding membership in this society shall be four dollars ($4.00) per annum.

Section 4. (a) Dues to this Society shall be payable to the treasurer of the constituent Society of the geographical area in which the member is employed, resides, or maintains his status as a student, or in the event no such constituent society exists, to the executive secretary of this Society.
(b) Annual dues for active, affiliate and corresponding members to this Society are payable on or before July 1 of each fiscal year.
(c) Dues for a student member may be renewed at the end of 12 months for another year, provided he has not in the interim become eligible for active membership in this Society.

Section 5. (a) Any active, affiliate, or corresponding member who cannot pay dues for the current fiscal year shall notify this Society by sending written notification direct to the executive secretary not later than September 1st of that year.
(b) Any active, affiliate, or corresponding member who fails to pay annual dues to this Society by September 1st shall have his name removed from the membership list.
(c) A person whose name has been removed from the membership list of this Society may be reinstated at any time provided he meets the requirements for membership of both his constituent society and this Society. Unless a person has previously resigned, to be reinstated in this Society he shall pay current annual dues to his constituent society with any back dues or reinstatement fee the constituent society may require, and current year's dues to this Society plus a reinstatement fee of two dollars to this Society.
(d) Any active or affiliate member who changes his residence or place of employment to another state, territory or nation shall become a member of the constituent society of his new residence or place of employment for the remainder of the fiscal year without further payment of dues.

Section 6. A new applicant elected to active, affiliate, or corresponding membership in this Society after January 1st and prior to May 1st shall pay one-half of the annual dues to this Society for the remainder of the fiscal year.

Section 7. An applicant elected to active, affiliate, or corresponding membership in this Society between May 1st and June 30th shall pay annual dues for one year which shall entitle him to all the privileges of his respective membership for a period up to fourteen months. He shall pay no further dues to this Society until the second fiscal year following the date of his application.

ARTICLE VIII
Officers

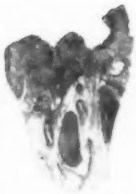
Section 1. The officers of this Society shall be president, president-elect, recording secretary and treasurer.
(a) The president shall be the chief executive of this Society and shall preside at all meetings of the Board of Directors and of the House of Delegates. He shall be an exofficio member of all standing and special committees except the Nominations Committee. He shall set up such standing committees as are provided for in the By-Laws except the Nominations Committee. He shall appoint with the approval of the Board of Directors, such special committees as are needed.

Section 2. The executive secretary shall contract for technical exhibits for the Annual Session of the Society and, working with the local chairman of exhibits, shall arrange for all technical exhibits. All receipts from technical exhibits shall go into the treasury of this Society.

Section 3. The Executive Secretary shall be included as a non-voting member of the Board of Directors and of the Advisory Council.

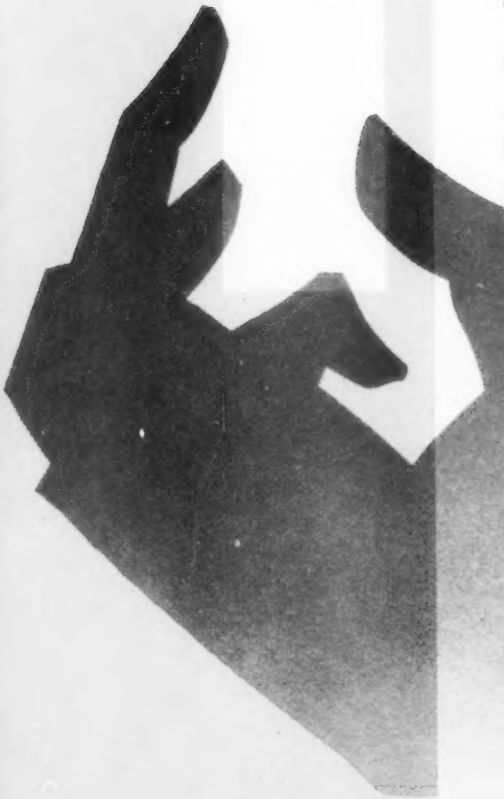
Section 4. The Executive Secretary shall distribute to the president, the president-elect and the other officers, and to the committee chairmen and to Advisory Council members, the names and addresses of the officers and committee chairmen of constituent societies as soon after October 1 as their membership status in this Society can be determined, and such lists prepared.

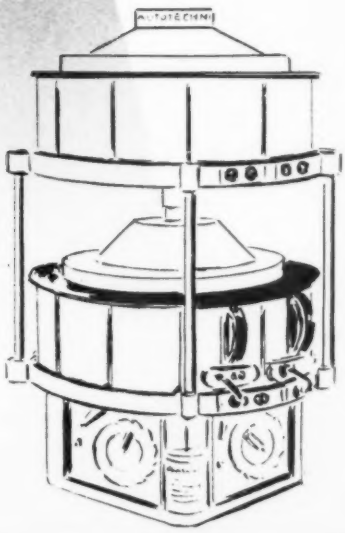
Substitute: pages 4, 5, and 6 for pages 4, 5, and 6 now in your copy—substitute sections above as noted on top of page 9, ARTICLE IX. Cut apart on dotted lines.



the *section* is the payoff

The better tissues you get with it are the ultimate proof of the Autotechnicon's worth. Processing is always dependably uniform, staining always diagnostically trustworthy. And you get finished tissues faster . . . thanks to the unique Autotechnicon principle of reciprocal displacement and controlled warming. There's a brochure that explains the why of all this. Let us send it to you.





Autotechnicon®

first name . . . and last word in histologic processing

THE TECHNICON COMPANY

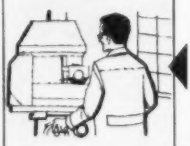
Chauncey • New York

Expert MICROTOME KNIFE RECONDITIONING

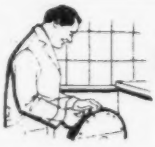
ALL MAKES • GUARANTEED EDGES • ALL SIZES

48 Hour Service is routine with our systematic . . .

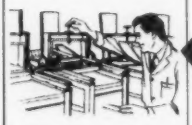
Receiving department carefully unwraps and records your instruments.



Grinding department where the sides of your knife are wet ground to a smooth, accurate finish.



Buffing and polishing department gives your knife that new appearance.



Honing department where those superb edges are produced on the Lipshaw automatic honer-stropper which is our invention.

Inspection department where the profile of every edge is inspected under a 100 x lens. Even a slight imperfection is rejected.

Shipping department. Here your knives are coated with oil, wrapped, cartoned and mailed.

STEP METHOD

Microtome knife reconditioning is accomplished in our plant by modern, automatic grinding and honing machines which were designed for microtome work only. Our operators have years of experience in this specialized field and understand the importance of microscopically true edges for cutting perfect tissues.

SIZE	PRICES
Knives up to:	
125 mm	4.50
185 mm	5.50
200 mm	8.00
250 mm	11.00
300 mm	12.00

MICROTOME KNIFE BACKS:
Stainless steel springbacks properly measured and adjusted to produce the exact angle for your microtome knife. Price two cents per mm.

Lipshaw MANUFACTURING COMPANY
7446 CENTRAL AVE. • DETROIT 10, MICHIGAN